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METHOD OF DETERMINING AGE OF BLISTER RUST INFECTION ON WESTERN WHITE PINE [1]

By H. G. LACHMUND [2]

Pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Blister rust (*Cronartium ribicola* Diet.) was discovered in western North America in the fall of 1921. In 1922 it was found that the disease ranged over a wide area in southwestern British Columbia and adjacent Washington. Apparently it was originally introduced near Vancouver in 1910.[3] Its range now extends practically from Alaska to California and from the Pacific coast in Washington east into Montana. The chief aecial host within this range is native western white pine (*Pinus monticola* Doug.). Investigations of the rust in this great new range have been carried on by the Division of Forest Pathology since 1922. One of the first essentials in these investigations was to establish a method of determining the age of infection on pines. In the present paper this problem is discussed and a solution offered.

Pinus monticola occurs in two distinct geographic belts. One of these belts extends between the Pacific coast and the eastern slopes of the Coast Range in British Columbia, from about 150 miles north of the international boundary, southward into northwestern Washington, and thence mainly along the Cascade Mountains of Washington and Oregon into the higher elevations of the Sierra Nevada of California; the other belt extends from about 200 miles north of the international boundary, in the interior of British Columbia, southward into the main commercial range of the species in northern Idaho and adjacent Washington and Montana. Until 1929 the known range of pine infection by the blister rust was confined chiefly to the coastal belt in British Columbia and northern Washington and to a small portion of the interior belt 120 to 135 miles north of the boundary, in British Columbia. The range of infection is now known to extend, in the coastal belt, south in the Cascade Mountains to central Oregon, and, in the interior belt, into the main commercial pine region of Idaho. The results reported here are mainly from studies made within the earlier known portions of the range, which constitute the area of longest established and heaviest infection, but they are also

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[2] The writer was assisted in the collection of data on which this article was based by J. L. Mielke, T. S. Buchanan, A. A. McCready, J. W. Kimmey, and W. F. Cummins, of the Division of Forest Pathology, and C. N. Partington, formerly of that division, and by W. V. Benedict, of the Division of Blister Rust Control.

[3] PENNINGTON, L. H. RELATION OF WEATHER CONDITIONS TO THE SPREAD OF WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Jour. Agr. Research 30: 598. 1925.

based on data from the newer sections of the range. The data from the two areas are in such close agreement as to indicate that the results will be applicable to the entire range of *P. monticola* in the Pacific Northwest.

CONDITIONS GOVERNING INFECTION OF WESTERN WHITE PINE

Cronartium ribicola infects its aecial hosts through the needles.[4] The needles of *Pinus monticola* are regularly held for three full growing seasons. Although some needles fall at the end of the third season, a great many are generally held through the fourth season, and some are commonly held to the end of the fifth season. If the growth rates of the needle-bearing parts and the environmental conditions are favorable, a few needles may be retained through the sixth or even the seventh season, and very exceptionally a needle bundle or two may persist through the eighth season. Thus infection may conceivably find entrance to the bark on internodes of any age up to eight seasons. It is obvious, on the other hand, that the best chances for entrance are on the younger growth and that the dropping of the needles prevents much infection from entering on internodes more than 4 seasons old.

Infection of the needles may occur at any time that moisture conditions are favorable during the period in which sporidia are produced. For all practical purposes, in the range of *Pinus monticola* this may be considered as limited to the period during which teliospores are produced; or, roughly, from about June 1, when these spores first begin to form, until about November 30, when Ribes plants have lost their leaves and most of the fungus spores have germinated.

Ordinarily the majority of the teliospores are produced within a relatively short period. This concentration of telial sporulation is particularly marked, and comes earliest in the pine-infection centers where Ribes plants are plentifully infected by aeciospores early in the season. Under these conditions the teliospores are usually produced in maximum quantities, and their production reaches its height in July or August.

Any favorable moist period during the time when teliospores are abundant results in the production of great numbers of sporidia and, if other conditions are favorable, in extensive infection on pines. On the other hand, if conditions are not favorable, the delicate sporidia, through ineffective germination or loss of viability, soon lose all power to infect, and thus the pine-infecting potentialities of the fungus on Ribes are largely dissipated for the season. The present investigations have shown that for the range of *Pinus monticola* in general conditions are favorable for heavy pine infection on an average of one season out of three.

DEVELOPMENT OF THE CANKERS

Infection is followed by an incubation period during which the rust grows through the needle into the bark, where its further development causes sufficient discoloration or swelling to permit of an accurate macroscopic diagnosis.[5] This period varies for individual infections.

[4] CLINTON, G. P., and MCCORMICK, F. A. INFECTION EXPERIMENTS OF PINUS STROBUS WITH CRONARTIUM RIBICOLA. Conn. Agr. Expt. Sta. Bul. 214: [428]–459, illus. 1919.
[5] CLINTON, G. P., and MCCORMICK, F. Op. cit., p. 449–455.

Observations of the rust through its western range on *Pinus monticola* indicate that the first discoloration or visible swelling of the bark that marks the incipient canker appears only under exceptional circumstances in the first season following that of infection, that it appears principally in the second season, to a lesser degree in the third season, and to a negligible degree, if at all, later than the third season.

The period required for production of the fruiting stages varies also.[6] When the incipient canker makes its appearance early or during the middle of the season, pycnia are generally formed upon it the same year. Cankers appearing toward the end of the season usually do not produce pycnia until the next year. On the majority of the cankers aecia are formed in the year following that in which pycnia are produced, but on many not until the second year.

For young cankers it is seldom difficult to determine the year's growth (wood [7]) on which they originated, and on thrifty trees the internodes of entrance can usually be determined for cankers of much greater age. The age of the growth on which the canker originated readily establishes the maximum possible age of the canker, since it is obvious that infection can not occur prior to the formation of the part infected. Considered in relation to the general appearance of the canker, this affords a rough index of its age. But in the great majority of cases it is impossible to determine accurately the year of infection for the individual canker. Only by considering large numbers of cankers classified according to the year's growth upon which they occur and to their stage of development can the year of infection be determined.[8]

CANKER PRODUCTION

Since the teliospores in pine-infection areas are usually produced within a relatively short period during any season, the occurrence of heavy infection of the pines will necessarily be correspondingly restricted in point of time, possibly to a few days toward the end of the period of teliospore production, or immediately following it. As might be expected, therefore, incipient canker formation in the infection centers generally occurs in definite waves, and usually the majority of the cankers of the individual waves show a marked tendency to form simultaneously.

Heavy waves of canker formation occurred in most of the infection-study areas of the coastal section in 1922 and 1923, in the interior section in 1924, and more or less generally throughout the range in 1929. In 1922 most of the cankers appeared in midseason, during late July and August. In 1923 the greatest numbers appeared in June and July. Most of those produced in the interior study areas in 1924 developed from midseason until September, although some continued to form until the end of the season. In 1929 concentrated production generally occurred toward the end of the season and in some places started up for a time the following spring.

[6] SPAULDING, P. INVESTIGATIONS OF THE WHITE PINE BLISTER RUST. U. S. Dept. Agr. Bul. 957:24–27. 1922.

[7] The term "wood" has been used by others in a sense similar to that for which the present writer prefers the term "growth," to designate internodes of given years.

[8] PENNINGTON, L. H. Op. cit., p. 596.

Most of the heavy waves of canker formation and some of less intensity have stood out distinctly, in some cases strikingly so. For example, at an infection area near Revelstoke, British Columbia, where the rust was discovered on pines in 1922 and had clearly been present on them for a number of years, no more than 48 cankers, incipient or otherwise, could be found on them until 1924. In June of that year a wave of incipient canker formation began, reaching such proportions in July and August that by September incipient and juvenile cankers could be counted in the area by the thousands. At another area near Revelstoke, where infection had been present on the pines since 1922, the number of cankers increased more than 1,000 per cent during the latter part of the growing season in 1929 and the spring of 1930.

DISTRIBUTION PATTERN OF CANKERS IN INCIPIENT CANKER WAVES

The incipient cankers of the waves of canker formation that occurred in 1922 maintained a highly regular numerical arrangement in their distribution on the internodes of different years. The youngest growth on which these cankers occurred was that of 1920. Most of the cankers, however, were concentrated on the growths of 1919 and 1918, the greatest number being on 1919 growth. Their occurrence was relatively light on 1917 growth, infrequent on 1916 growth, and very exceptional or lacking on older growths. Since these incipient cankers had appeared almost simultaneously, it seemed probable that the majority, at least, had resulted from a common infection year.

Inasmuch as some of the cankers occurred on 1920 growth, it was evident that the majority must have resulted from an infection not earlier than that of 1920. Since none was present on growth younger than 1920, this year was evidently the infection year. If the infection had occurred in 1921 some of the cankers would undoubtedly have been present on 1921 growth; if the infection had occurred in 1922, there would not have been sufficient time for even incipient cankers to develop, or assuming incubation to have taken place, the cankers would have appeared on the growth of 1922 as well as on that of other years.

The waves of canker formation that occurred in 1923 showed a distribution closely analogous to that of the 1922 waves. No cankers were found on 1923 or 1922 growth. Relatively few were found on 1921 growth; the great majority occurred on 1920 and 1919 growth, and the greater number of these on 1920 growth; 1918 growth had relatively few, and older growths were very little affected. The same general relations have characterized the distribution of cankers of each distinct wave of canker formation since then.

It is evident that this characteristic numerical distribution of the cankers, combined with similarity in stage of development, provides a means of ascertaining the infection years for groups of cankers in more advanced stages, thus making it possible to determine the main years of infection and to work out the history of the rust from analyses of older classes of cankers.

For convenience in presenting and summarizing the observations and data the following symbols are used:

a, youngest growth upon which cankers of the infection wave were found.
b, growth 1 year older than *a*.
c, growth 2 years older than *a*.
d, growth 3 years older than *a*.
e, growth 4 years older than *a*.
f, growth 5 years older than *a*.
g, growth 6 years older than *a*.
h, growth 7 years older than *a*.

Figure 1 shows diagrammatically these symbols and the normal occurrence of needles for several successive years.

Most of the needles on *d* in A are dropped at the end of the season, and the remainder are generally dropped at the end of the season the following year. The chances of infection through the needles on

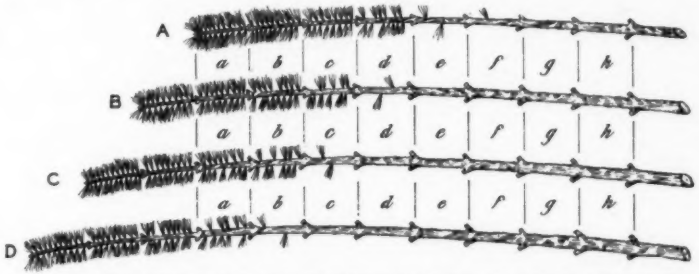

FIGURE 1.—Needle retention in *Pinus monticola* infected with *Cronartium ribicola:* A, Branch after completion of internode *a;* B, branch one year later; C, branch two years later (when most of the cankers appear); D, branch three years later (when the majority of the remaining cankers appear); *a*, youngest growth, or internode, on which cankers of the infection wave were found; *b* to *h*, growths, or internodes, 1, 2, 3, 4, 5, 6, and 7 years older than *a*, respectively

older growths are obviously small. Undoubtedly this accounts for the small representation of cankers on such growths shown in the characteristic pattern of the cankers of the distinct waves of canker formation, which is summarized below:

Growth	Cankers	
a	Relatively few.	
b	Greatest number	large majority.
c	Considerable numbers	large majority.
d	Relatively few.	
e	Very few.	
f	Exceptional.	
g	None.	
h	None.	

Sample counts of cankers from these waves are presented in Table 1; the number on each year's growth is expressed as a percentage of the total.

The individual records consistently show the same general relationships. The percentage of cankers on *a* is normally relatively small, averaging about 10; *b* and *c* together have consistently the largest percentage (80–90), with much the largest percentage (usually more than 50) on *b;* the percentage on *d* is generally relatively small (averaging about 5); and that on older growths is practically negligible.

TABLE 1.—*Canker records from distinct waves of canker formation*[a] *in various areas*

COASTAL REGION, BRITISH COLUMBIA

No.	Elevation	Location	Youngest growth showing cankers (a)[b]	Year of canker formation	Number of cankers	Percentage of cankers on—						
						a[c]	*b*	*c*	*d*	*e*	*f*	*g*
			1920	1922	77	17	52	23	7	1	0	0
			1920	1922	53	10	58	28	4	0	0	0
			1920	1922	68	6	50	43	1	0	0	0
			Total or average		198	11	53	31	4	1	0	0
			1921	1923	76	12	63	13	9	3	0	0
			1921	1923	81	14	60	20	6	0	0	0
1		Thurston Bay	1921	1923	136	7	57	34	2	0	0	0
			Total or average		293	10	59	24	5	1	0	0
			1922	1924	123	19	61	17	3	0	0	0
			1924	1926	115	9	55	34	1	1	0	0
			Total or average		729	12	57	26	4	1	0	0
2		Pender Harbor	1921	1923	134	5	54	37	4	0	0	0
			1920	1922	40	8	41	35	8	0	0	0
	Near sea level		1921	1923	417	8	49	37	6	0	0	0
			1921	1923	302	10	53	32	5	0	0	0
3		Chee Kye	Total or average		719	8	51	35	6	0	0	0
			1922	1924	48	17	64	19	0	0	0	0
			1923	1926	45	16	60	22	2	0	0	0
			Total or average		852	9	52	33	6	0	0	0
4		Sperling	1921	1923	91	7	55	31	7	0	0	0
			1923	1925	56	11	57	30	2	0	0	0
			1924	1926	112	7	68	21	4	0	0	0
5		Qualicum Beach	1927	1929	40	12	55	30	9	0	0	0
			Total or average		208	9	63	25	3	0	0	0
			Total or average		2,014	10	55	30.2	4.6	.2	0	0

COAST MOUNTAINS, BRITISH COLUMBIA

	Feet											
6	1,300	Daisy Lake	1921	1923–24	98	10	56	24	10	0	0	0
			1923	1925	145	2	53	43	2	0	0	0
			1924	1926	123	4	60	33	2	0	0	0
7	800	Owl Creek	1927	1929	52	8	48	34	10	0	0	0
			Total or average		320	4	55	38	3	0	0	0
			1920	1922	121	14	42	33	10	1	0	0
			1921	1923	124	13	46	34	4	1	0	0
8	1,300	Mile 72, P. G. E. Ry	Total or average		245	13	44	35	7	1	0	0
			Total or average		663	8	51	34.5	6	.5	0	0

[a] From main body of wave where possibility of admixture of cankers of two successive years was least in each case. Samples were taken indiscriminately through the stand from trees that could be examined from the ground or easily climbed. Cases indeterminate in regard to age of growth infected or stage of development of the canker were not included.

[b] From sample records of the Division of Blister Rust Control.

[c] For definition of symbols *a*, *b*, *c*, *d*, etc., see page 679.

TABLE 1.—*Canker records from distinct waves of canker formation*[a] *in various areas*—Continued

OTHER AREAS

No.	Elevation	Location	Youngest growth showing cankers (*a*)[b]	Year of canker formation	Number of cankers	Percentage of cankers on—						
						a[b]	*b*	*c*	*d*	*e*	*f*	*g*
9	1,500	Revelstoke, British Columbia.	1922	1924	296	7	52	31	8.5	1.5	0	0
			1922	1924	307	3	51	34	10	2	0	0
			1922	1924–25	180	14	52	27	6.5	.5	0	0
			Total or average		783	7	52	31	8.7	1.3	0	0
10	2,000	Columbia River, 8 miles north of Revelstoke.	1927	1929–30	1,805	12	54	30.12	3.5	.33	.05	0
11	1,700	Arrow Park, British Columbia.	1927	1929	135	8	53	36	3	0	0	0
12	3,100	Apex, British Columbia, experiment plot.	1928	1930	144	5	52	32	10	1	0	0
13	2,500	Mount Hood, Oreg., near Rhododendron.	1927	1929	92	9	42	37	12	0	0	0
14	500	Eagle Creek, Oreg.	1927	1929–30	243	14	42	36	7	1	0	0
			Total or average		3,202	10.2	52.3	31.3	5.5	.67	.03	0
			Total or average		5,879	9.8	53.2	31.3	5.21	.47	.02	0

See footnotes on p. 680.

DETERMINATION OF YEAR OF INFECTION

The typical distribution pattern of the incipient cankers, their simultaneity of appearance, and the conditions governing infection of the pines may be taken as conclusive evidence that the majority of the cankers of each wave are approximately the same age and of common origin; i. e., that they resulted from an infection of the same year. On this basis, the year of infection for each of these waves is determined by the youngest growth affected. In most cases this is the growth that formed two seasons before the appearance of the cankers. In the waves that became apparent in 1922, for example, the youngest growth showing cankers was that of 1920; in the waves of 1923, it was the growth of 1921; in the 1924 waves, it was the growth of 1922, etc.

Since there may be some doubt as to these conclusions, however, the analysis of the 1922 waves may be carried somewhat further. It may be assumed that infection occurred in 1921. Since none of the cankers were present on 1921 growth, it would follow either that the needles of 1921 were immune to infection or that a longer time was required for incubation in the needles and bark of 1921 than in the needles and bark of older growths, and that the infection on 1921 growth had not yet become apparent. Since relatively few of the cankers occurred on 1920 growth, it would be necessary to assume either that the needles of 1920 were resistant to infection in 1921 or that incubation was slower for this growth than for older growths. In accordance with the regular habit of needle retention in *Pinus monticola* (fig. 1), 1919 growth (*b*), which was in its third season of development in 1921 (fig. 1, B), lost some of its needles at the end of the season in 1921. Most of the needles that remained on 1918 growth (*c*) fell at the same time. Yet the great majority of the

cankers occurred on 1919 and 1918 growths. Therefore, for 1918 growth at least, the rust must have grown through the needles and into the bark with extraordinary rapidity. The growth of 1917 (*d*) was practically devoid of needles in 1921. (Fig. 1, B.) Yet the number of cankers on this growth, although small in comparison to the number on 1919 and 1918, growth was actually very considerable. Since most of the few needles remaining on this growth were cast in the fall of 1921, the only possible explanation would be that some of the infection on this growth occurred directly through the bark, which was then 4 seasons old. Certainly this explanation is too improbable to merit further consideration, and it is obvious that to assume that infection occurred in 1922 would require an explanation still more improbable.

If it is assumed that the cankers of the 1922 waves resulted from the combined infection of two or more years, it would be necessary to conclude, since these waves exhibited the typical arrangement of distinct canker-formation waves generally, that this arrangement characterized the distribution of cankers of two or more infection years forming simultaneously and that all the distinct waves arose from similar combinations, for the typical arrangement is too consistently maintained to be accidental. On this basis, for example, if it is assumed that the 1922 waves resulted half from 1919 infection and half from 1920 infection, then the 1923 waves must have originated from equal proportions of infection of 1920 and 1921, the 1924 waves from equal proportions of infection of 1921 and 1922, and the 1929 waves from equal proportions of infection of 1926 and 1927. In other words, the infection years would necessarily bear a regular proportional relationship toward one another, which is obviously beyond probability.

Evidently, then, it is reasonable to accept the typical arrangement as evidence that the majority of the cankers of any one of these waves resulted from infection in a single year and that the youngest growth affected (*a*) marks the infection year; i. e., it is the growth formed the year infection occurred. Thus Table 1, column 4, gives the infection year for each of the waves of which the cankers were counted.

The dates of the infection as determined by the foregoing methods have been further confirmed by observations of weather conditions. In the years shown by the analyses of cankers to have been heavy infection years, conditions have been favorable for heavy pine infection; and when analyses have shown that the infection was light, conditions have been unfavorable for infection.

The method of age determination is thus supported by a complete chain of evidence comprising the following series of phenomena found occurring repeatedly and uniformly throughout a wide range of representative areas over a period of nine years of observations:

(1) Evidence or actual observations of outstanding favorable seasonal conditions for infection of the pines.

(2) Two seasons later a heavy wave of canker formation, sometimes extending into the third season.

(3) Distribution of these cankers in accordance with a characteristic distribution pattern.

(4) Only one acceptable explanation of the canker distribution, namely, that most of the cankers in each wave were the same age; i. e., that they resulted from an infection of the same season.

(5) Date of the infection season fixed by the distribution of the cankers as a favorable season two or three seasons before the appearance of the majority of the cankers.

Since, however, before the appearance of the canker the infection year is indicated only by its outstandingly favorable conditions in sporidia production and moisture, the evidence for the hypothesis herein advanced is primarily circumstantial. It is desirable, therefore, to consider cases where there could be no doubt in regard to the year of infection. The opportunity to do this occurred recently in a case in which the infection year was definitely known before the cankers appeared. In this case two Ribes plants, the only specimens standing in a small isolated area of sound pine, were infected artificially in 1928. Ribes infection was not present in the area in 1927 or in 1929. In the growing season of 1930, 144 incipient cankers appeared only on trees within a short distance of the Ribes and in greatest profusion on those in the immediate vicinity of the Ribes. There can be no doubt that these cankers resulted from the infection on the Ribes plants in 1928. The record of these cankers (Table 1, area No. 12) follows closely the general trend and average of the other records.

It has been stated earlier in this paper that cankers are rarely found on trees in the first season after the year of infection. This is the case with most trees. On trees more than 3 feet high and 8 years old the writer in nine seasons of observations has never seen an incipient canker on growth younger than that formed two years before the year of examination. On the youngest seedlings and on those up to about 5 years old, however, the writer has positive proof that a great many if not most of the cankers appear in the season following that of infection. In determining the heavy infection years, therefore, cankers on such seedlings must not be included with the records of infection on the older trees, which generally constitute most of the growth in any natural infection area and therefore have the great majority of the cankers.

Variation in needle retention is another factor that must be taken into account in investigations of the history of infection in any pine area. The normal period of needle retention described earlier in this paper does not apply absolutely, for in areas where growth is relatively slow, usually at higher elevations, the needles are commonly held for a somewhat longer period than is normal; and where growth is particularly rapid they are actually held for a somewhat shorter period than is normal. This is especially noticeable on the faster growing parts such as the leaders and upper branches of thrifty trees, where the needles are commonly shed a full year earlier than is normal.

Since infection occurs through the needles, it is clear that the distribution of the cankers on growths of different years will be influenced by differences in needle retention. It may be assumed, for example, that areas 8, 9, 12, and 13, in Table 1, represent conditions of high altitude and slow growth and that areas 1 and 5 represent conditions of lower altitude and particularly rapid growth. Table 2 shows the two scales of arrangement for the two types of conditions separately. The arrangement for the grand total of Table 1 may for all practical purposes be considered normal; the data are given as approximate percentages for comparison in Table 2.

TABLE 2.—*Percentage of cankers found on growths of different years under conditions of low altitude and rapid growth and of high altitude and slow growth*

Growth affected	Percentage of cankers under indicated conditions			Growth [a] affected	Percentage of cankers under indicated conditions		
	Low altitude (rapid growth)	High altitude (slow growth)	Normal		Low altitude (rapid growth)	High altitude (slow growth)	Normal
a [a]	10+	7+	10−	*f*	0	0	Trace.
b	60	40+	53+	*g*	0	0	0
c	26−	34−	31+	*h*	0	0	0
d	3+	9+	5+	*i*	0	0	0
e	0.5	1−	0.5−				

[a] Growth of season of infection; i. e., youngest growth showing cankers of the wave.

As might be expected, the high-altitude group shows a greater spread of the infection to older growths, *c* and older growths having more than the normal amounts and *a* and *b* growths less; whereas the low-altitude scale shows more infection than normal on *b* growth and less than normal on *c* and older growths. As a rule, the differences are small, and the same general relationships prevail in all the scales.

TECHNIC FOR DETERMINING AGE OF CANKERS

The application of the foregoing scales of arrangement is simple. On encountering a new infection area the investigator first determines the general span of needle retention in order to decide what scale to apply in determining the infection years. The normal scale or even the typical distribution as expressed in descriptive terms alone (p. 679) will usually suffice for general application. Records should then be made of the cankers classified according to stage of development. The following classification has been found most useful:

Young cankers:

First symptoms.—First signs of infection on the bark; incipient cankers indicated by discoloration less than one-half inch in length on the smaller, slower-growing twigs and branches and up to about three-quarters inch in length on the larger branches and leaders, usually showing little or no swelling.

Juvenile.—Small infections that have developed beyond the stage of first symptoms but have not yet produced pycnia.

First pycnia.—Infections that at the time of the count or earlier in the same season were bearing pycnia for the first time.

Pycnial scars.—Infections that had borne pycnia for the first time the preceding year but failed to produce aecia in the spring of the year in which the count was made.

First aecia.—Infections that bore aecia for the first time in the spring of the year in which the count was made.

Old cankers:

Aecia produced twice.—Cankers that bore aecia for the second season in the spring of the year in which count was made.

Aecia produced several times.—Cankers that had borne aecia for several seasons.

Dead.—Old cankers in which death prevented further development.

From first to last, these headings represent normal progressive steps, following incubation, in the development of the individual infection.

In order to apply this scheme of classification it is necessary to consider two facts: (1) That cankers grow longitudinally at an average rate of approximately 2.5 to 3 inches per year on twigs and branches less than one-quarter inch in diameter, and (2) that this rate increases

up to an average of about 7 or 8 inches a year on parts 3 inches or more in diameter. It is also necessary to be familiar with the normal development of fruiting stages described on page 677. Where fruiting has obviously been retarded, the plan of classification may be enlarged to include such cankers.

In Table 3 an illustration is given of the application of the distribution pattern to the analysis of the history of the rust in a typical infection area near sea level. Table 3 gives a count taken indiscriminately on April 27, 1924, from the cankers found on thrifty trees ranging from approximately 10 to 30 years of age. The count included only cankers for which it was possible to determine definitely the age of the growth on which the canker started and the stage of development of the canker.

TABLE 3.—*Canker tabulation, Chee Kye, British Columbia, April 27, 1924*

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
						Aecia produced		Dead
	First symptoms	Juvenile	First pycnia	Pycnial scars	First aecia	Twice	Several times	
1	2	3	4	5	6	7	8	9
1924	0	0	0	0	0	0	0	0
1923	0	0	0	0	0	0	0	0
1922	0	0	0	0	0	0	0	0
1921	12	9	0	4	19	0	0	0
1920	19	21	0	23	162	3	0	0
1919	2	10	0	16	126	20	0	0
1918	0	0	0	0	27	14	2	0
1917	0	0	0	0	0	3	2	0
1916	0	0	0	0	0	0	0	0
1915	0	0	0	0	0	0	2	0
1914 and older	0	0	0	0	0	0	0	0

The count did not include cankers of the first year of infection on pines in this area. Special search for older cankers showed, on growth ranging from that of 1910 to that of 1913, a few very old cankers. The arrangement of these old cankers and the fact that weather records indicate 1913 to have been an outstandingly favorable year for infection of both Ribes and pine, showed that the cankers probably resulted from infection in 1913. The analysis of the older cankers, not included in Table 3, showed considerable intensification in 1917 and 1918, particularly in the latter year. A comparison of the normal distribution pattern with the tabulation in Table 3 shows that much intensification occurred in 1920 and a very large amount in 1921. From the distribution relationships of the cankers listed in columns 2 and 6 of Table 3 it is evident that the great majority are of 1921 origin. The proportion of these cankers to older cankers, in view of the fact that the number recorded represents but an infinitesimal fraction of the number present, indicates that a great wave of intensification occurred in 1921. Practically all the cankers in column 7, from their arrangement, are clearly of 1920 origin.

Table 4 gives a tabulation for the same area from November 15 to 16, 1924, the year in the spring of which the tabulation in Table 3 was made. The same rules applied in making the tabulation given in Table 3 were observed in making the tabulation given in Table 4.

TABLE 4.—*Canker tabulation, Chee Kye, British Columbia, November 15 to 16, 1924*

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
	First symptoms	Juvenile	First pycnia	Pycnial scars	First aecia	Aecia produced		Dead
						Twice	Several times	
1	2	3	4	5	6	7	8	9
1924	0	0	0	0	0	0	0	0
1923	0	0	0	0	0	0	0	0
1922	6	1	3	0	0	0	0	0
1921	22	8	21	0	29	0	0	0
1920	4	5	24	22	137	8	0	0
1919	0	0	2	14	83	19	0	0
1918	0	0	0	1	16	7	3	2
1917	0	0	0	0	0	0	13	0
1916	0	0	0	0	0	0	6	0
1915	0	0	0	0	0	0	1	0
1914	0	0	0	0	0	0	1	0
1913 and older	0	0	0	0	0	0	0	0

A comparison with the normal distribution pattern shows clearly that considerable additional intensification of infection on pines occurred in 1922. The cankers in columns 2 to 4 of Table 4 are mainly of 1922 origin. Their number is small in proportion to the number of 1921 cankers (columns 5 and 6), but they seem to exceed the number in 1920 (column 7). It must be remembered, however, that as the cankers grow older they are increasingly difficult to classify exactly according to stage of development and year of origin. Therefore, since doubtful cases are not included in the counts, some allowance must be made to balance this difference. Observations indicated that cankers of the 1920 wave were slightly more abundant than those of the 1922 wave. In view of the fact that aecial sporulation and the opportunities for the infection of Ribes were much better in 1922 than in 1920, owing to the development of sporulating cankers between these years, it was evident from the count, together with the observations, that 1920 was a considerably more favorable year than 1922 for infection in this area.

As already indicated, canker formation in *Pinus monticola* takes place chiefly in the second year after the year of infection and to some extent in the third year. The cankers generally begin to form in the spring of the second year and are produced in the greatest numbers during the summer and fall of that year. In the winter, growth practically ceases in the rust as well as in the host. Consequently, if all the cankers have not made their appearance in the second year, the remainder do not ordinarily develop until after active growth is resumed the following spring.

Normally the proportion of cankers produced in the third year is small and the period of formation is confined to the first months of spring. Frequently, however, a considerable number, sometimes many, are produced, and the period of formation continues until well into the summer.

Where successive infection years occur, the third-year production of cankers frequently results in an overlapping in the formation of the cankers of one infection year with that of the next and the intermixture of incipient cankers of two infection years. Since the proportion of cankers produced in the third year is usually small and production is generally limited to spring, the overlapping and intermixture are correspondingly slight and limited to spring; but when third-year production is considerable and prolonged the overlapping and intermixture may be extensive.

Besides occurring at incipiency, intermixture of cankers normally takes place during the later stages because of variation in rate of development and production of fruiting stages and the consequent impossibility of classifying the cankers according to the year of origin after they have passed the early stages. Thus, cankers formed in the fall of one year do not produce pycnia until the following summer. Meantime cankers resulting from the next infection year are formed in the spring and produce pycnia at the same time as do the cankers formed the preceding fall. The result is an intermixture of the cankers of the two consecutive infection years in the "First pycnia" column. If the count is taken shortly before the formation of pycnia, there will be an intermixture of the two sets under the "Juvenile" classification.

The frequency of intermixture increases with increasing age. For example, not all the cankers that produce first pycnia one year produce aecia the following spring; some cankers normally produce their first aecia the second spring after the production of pycnia. Where successive infection years occur this will result in considerable intermixture under the classification "First aecia." The extent and complexity of intermixture will obviously be still greater among the older cankers.

This intermixture, whether it occurs at incipience or later, tends to obscure the actual distribution relationships of the cankers. Fortunately, the infection years in the earlier stages of the epidemic are generally separated by periods of one to several years during which little or no infection occurs; if later they become successive they usually are highly unequal, so that the years of heavy infection stand out. But even if intermixture is extensive the infection years may be differentiated by use of the typical distribution relationships.

The application of the relationships in these cases may be best illustrated by a hypothetical example. Assuming that 100 cankers of each of two successive infection years, 1922 and 1923, represent a single stage of development and that in both cases the distribution of the cankers is identical with the normal scale, it follows that the distribution of each set and of the sums of the two sets, the latter constituting the numbers recorded, would be approximately as given in Table 5.

TABLE 5.—*Hypothetical distribution of cankers in a case of intermixture of 100 cankers from each of two infection years*

Growth affected	Number of cankers			Growth affected	Number of cankers		
	From 1923 infection	From 1922 infection	Total		From 1923 infection	From 1922 infection	Total
1923	10	0	10	1918	0	1	1
1922	53	10	63	1917	0	0	0
1921	31	53	84				
1920	5	31	36	Total	100	100	200
1919	1	5	6				

Application of the scale to the combined totals in this case would permit an almost exact segregation of the two sets. Obviously such an equal combination and exact agreement of the individual components with any distribution scale is not to be expected in nature. But as already shown, there is consistently a sufficiently close adherence to the general relationships of the typical distribution pattern to permit analysis where intermixture occurs.

The detailed data collected by the Division of Blister Rust Control on the Long Meadow Creek and Three Bear Creek infection area, Idaho, in July, 1930, represent an extensive admixture caused by overlapping of canker formation and by variation in development, and illustrate a practical application of the distribution pattern to actual conditions. In this case the overlapping of canker formation and consequent intermixture in the cankers of the younger stages were extreme. (Table 6.) The area may be classified as normal in general conditions. But since most of the trees were of considerable height and the majority of the cankers were situated on the slow-growing branches of the lower crowns, which formed the major portion of the substratum for infection in close proximity to the Ribes, the scale of arrangement for slow-growing conditions was used in the analysis of the data.

TABLE 6.—*Canker tabulation, Long Meadow Creek and Three Bear Creek infection area, Idaho, July, 1930*

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
						Aecia produced		Dead
	First symptoms	Juvenile	First pycnia	Pycnial scars	First aecia	Twice	Several times	
1	2	3	4	5	6	7	8	9
1930	0	0	0	0	0	0	0	0
1929	0	0	0	0	0	0	0	0
1928	60	127	2	4	1	0	0	1
1927	111	750	35	61	65	0	0	3
1926	125	1,328	123	232	248	2	2	2
1925	39	578	73	146	304	6	7	2
1924	4	88	16	54	101	24	10	7
1923	0	12	1	10	11	4	7	3
1922	0	1	0	1	0	0	4	4
1921	0	0	0	0	0	0	0	1
1920 and older	0	0	0	0	0	0	0	0

A detailed analysis of the data in Table 6 gives the following results: The distribution of the cankers in column 2 indicates that they are primarily of 1928 origin, although the inclusion of a certain proportion originating from 1927 infection is suggested by the relatively large representation on 1926 growth. Column 3 contains the majority of all the cankers counted. A superficial examination of the figures indicates that many of the cankers originated from infection in 1928 but that the greatest number originated from infection in 1927. This may be seen by comparing the great numbers of cankers on 1926 and 1925 growths with the typical distribution pattern as expressed in descriptive terms alone (p. 677). A more critical analysis may be made by trial and comparison of assumed values on the basis of the relationships shown in the scale. In column 3 the 127 cankers on 1928 growth are obviously of 1928 origin. Assuming the occurrence of cankers of this year on older growths in this column to be approximately 610 on 1927 growth, 500 on 1926 growth, 100 on 1925 growth, and 10 on 1924 growth, a total of 1,347 cankers is obtained, leaving a remainder of 1,537 distributed as follows: 140 on 1927 growth, 828 on 1926 growth, 478 on 1925 growth, 78 on 1924 growth, 12 on 1923 growth, and 1 on 1922 growth. In terms of percentage the distribution for the 1928 group becomes: *a*, 9; *b*, 45; *c*, 37; *d*, 8; *e*, 1; and for the 1927 group: *a*, 9; *b*, 54; *c*, 31; *d*, 5; and *e*, 1. The figures show that about 82 per cent of the cankers in the 1928 group and 85 per cent of the cankers in the 1927 group occurred on *b* and *c* growths, which compares well with the normal pattern and at the same time balances the variations from the values of the high-altitude scale as closely as is necessary for practical purposes. The conclusion is the same as that derived from the superficial analysis; i. e., that many of the cankers originated from infection in 1928 but that the greater number originated from infection in 1927.

In column 4 of Table 6 it is evident that the great majority of the cankers originated in 1927, although a few of 1928 origin are included also. Since 2 of the cankers occur on 1928 growth, this column, in accordance with the high-altitude scale of arrangement, should contain about 14 cankers of 1928 origin on 1927 growth, 10 on 1926 growth, and 2 on 1925 growth. This makes a total of about 28 cankers of 1928 origin, leaving a balance of 222 distributed as follows: 9+ per cent on 1927 growth, 51+ per cent on 1926 growth, 32+ per cent on 1925 growth, 7+ per cent on 1924 growth, and less than 1 per cent on 1923 growth. A comparison of the high-altitude scale with the distribution pattern of these cankers shows an almost exact agreement of the figures with the scale and definitely determines 1927 as the infection year for the group.

In column 5 of Table 6 is found a class of cankers that made their appearance and produced pycnia in the year preceding that of the tabulation but failed to produce aecia in the tabulation year, 1930. It will be noted that four cankers in this column were found on 1928 growth. These cankers, therefore, originated from 1928 infection and made their appearance in the following season, 1929. Since this is extremely unusual, except on young seedlings up to about 5 years old, it is presumed that these cankers were recorded from such seedlings, of which undoubtedly there was a certain representation on the area. The distribution of cankers resulting from a given year's infection on such young trees differs somewhat from the normal distribu-

tion on older trees and averages about 15 per cent on *a* growth, 69 per cent on *b*, 14 per cent on *c*, and 2 per cent on *d*. According to this relationship, since 4 of the cankers occur on 1928 growth column 5 should contain about 18 such cankers on 1927 growth and 4 on 1926 growth, making a total of about 26 cankers that may be considered to have originated from infection of 1928. There remain 482 cankers, distributed as follows: 9 per cent on 1927 growth, 47+ per cent on 1926 growth, 30+ per cent on 1925 growth, 11+ per cent on 1924 growth, 2+ per cent on 1923 growth, and less than 1 per cent on 1922 growth. Comparison of the arrangement of these cankers with the high-altitude scale shows that the infection year was 1927.

The majority of the cankers in column 6 of Table 6 are of 1927 origin, although a few are of 1928 origin, and there is evidently a very considerable proportion of 1926 origin on 1926 and older growths in this column. This is clear not only from the arrangement of the cankers but from the fact that in general the production of aecia occurs only in part of the cankers in the year following that of their appearance, aecial production on most of the remainder occurring in the following year.

The distribution of the cankers in column 7 of Table 6 indicates infection in both 1926 and 1925. The number of 1926 cankers in column 7 is only a fraction of the number in column 6. Evidently, then, most of the cankers that originated from 1926 infection either failed to produce aecia in 1929, following the production of first pycnia in 1928, or did not reach the stage of pycnial production until 1929, or both, and did not arrive at the stage of aeciospore production until the spring of 1930.

The distribution of the cankers in column 8 of Table 6 indicates infection in both 1926 and 1925. If the 1926 cankers in column 8 had actually produced aecia more than twice it would appear that they must have formed and produced pycnia at least by 1927, the year following infection. As already stated, this is a rare occurrence except on very young trees. Since the proportion of such cankers in this case appears to be rather large and since at the time of year the count was made cankers that have produced aecia only twice may easily appear to have produced aecia more than twice, it is more probable that part of the 1926 cankers in column 8 had actually produced aecia only twice and should have been included in column 7.

The prevalence of cankers on 1924 growth, as shown in columns 7 and 8 of Table 6, indicates that the larger proportion originated from infection in 1925. The number of 1926 cankers recorded in column 6, however, is greater than the total of all cankers in columns 7 and 8 and, together with the 1926 cankers in these two columns, indicates a decidedly heavier infection in 1926 than in 1925.

From a recapitulation of the foregoing analyses and from a broader consideration of the data in Table 6, two years—1927 and 1928—stand out as years of very heavy infection, 1927 showing much the heavier infection. In considering the degree of infection indicated by the record for the year 1928, however, it should be remembered that part of the cankers do not become apparent until the third year after the infection year. The tabulation was made in 1930. The incidence of cankers of 1927 origin in the youngest classes in stage of development indicates that in the Long Meadow and Three Bear

Creek area there is a considerable lag in incubation and that relatively large proportions of the cankers may not make their appearance there until the third year after the infection year. On this basis, a considerable proportion of the cankers of 1928 origin might not appear until 1931. Therefore, since the record was made in 1930, the indications of the degree of infection in 1928 must necessarily be considered incomplete.

In addition to the infection of 1927 and 1928, the analysis shows a certain amount of infection in 1925 and 1926, the greater amount in 1926.

The earlier infection history of the area had to be studied by means of special counts of the oldest cankers that could be found. The arrangement of these cankers and their situation on the area indicated that an appreciable amount of infection occurred in several local foci in 1923. No cankers were found that could be assigned to any earlier infection year. The infection in 1923 and that in 1925, when the 1923 cankers were just making their appearance but had not yet produced aecia, was considerably heavier and more profusely distributed than is normal through infection of Ribes by the long-distance spread of aeciospores from the outside. The usual ratio of Ribes infection to that on pines indicated that at least one or two sporulating cankers were present in the near vicinity at the time. It seems probable, therefore, that the original invasion of the locality by the rust must have occurred some years earlier than 1923.

From the data secured by these special counts and from the analysis of the tabulation in Table 6 the infection history for this locality may be summarized as follows:

(1) Original invasion probably a few years before 1923.
(2) 1923.—Very few aeciospores; favorable infection season; infection of pines at several points in the area.
(3) 1924.—Very few aeciospores; unfavorable infection season; negligible intensification on pines.
(4) 1925.—Very few aeciospores; favorable infection season; appreciable intensification of infection on pines.
(5) 1926.—Great increase of aeciospores resulting from sporulation of the cankers of the infection of 1923; unfavorable infection season; considerable intensification on pines.[9]
(6) 1927.—Great majority of 1923 cankers produced aeciospores; aeciospore production probably doubled or trebled that in 1926; very favorable infection season; great intensification on pines.
(7) 1928.—Another large increase of aeciospore production as a result of beginning of sporulation on cankers of 1925 origin; favorable infection season; further heavy intensification on pines.

From the foregoing analysis it is evident that the application of the relationship of arrangement is essential to any determination of infection years where cankers of successive infection years are mixed. Pennington [10] has stated that—

when a large number of cankers of approximately the same stages of development are found distributed through the internodes of three or four successive years, it is practically certain that infection resulted from exposure in the last of these years.

[9] As a result of the beginning of sporulation of the cankers of 1923 origin the number of aeciospores produced on the area must have been increased many hundredfold over that in 1925. Although the intensification of infection of pines in 1926 was about five or six times greater than that of 1925, the fact that it was much less than might have been expected from the increase of aeciospores and the opportunities for infection of Ribes on the area indicates that infection conditions in 1926 were unfavorable.

[10] PENNINGTON, L. H. Op. cit., p. 596.

The application of the method of determining the age of the cankers, as reported in this paper, to similar distributions shows that in general this is true; but one would be at a loss on this basis to determine the infection years when, as is commonly the case, the distribution normally covers internodes of five years or when the cankers of two infection years are mixed. Even when the cankers found are confined to growths of only four years, such mixtures may occur, as is indicated from the analysis of column 7 of Table 6. A comparison with the distribution pattern is therefore necessary for accurate diagnosis in any case. Where the infection years are as badly mixed as those in Table 6, such a comparison constitutes the only means of differentiation. With its aid it is possible, even in such cases, to obtain a good picture of the heavy infection years and of the history of the progress of the rust on pines.

SUMMARY

Pinus monticola, in its natural range in the Pacific Northwest, generally casts most of the needles of any one year at the end of the fourth season, although some are held five seasons and a few may persist for as long as eight seasons. Since *Cronartium ribicola* infects its aecial hosts through the needles, infection in any season may conceivably find entrance to the bark on internodes up to 8 seasons of age but will necessarily enter chiefly on those of the last four seasons.

Conditions favorable for heavy pine infection in the Pacific Northwest are generally limited to a short period in any season and occur, on an average, about one season out of three. Heavy infection of pines in any year and locality usually becomes manifest in a more or less distinct wave of incipient canker formation in the second season following the season of infection. The cankers of any year of infection show a highly characteristic numerical relationship in their proportional distribution on the growths, or internodes, of different years. The characteristic relationship may be summarized as follows:

Growth affected	Number of cankers	
a. Growth of the season of infection	Relatively few.	
b. Growth 1 year older than *a*	Greatest number	large majority.
c. Growth 2 years older than *a*	Considerable number	large majority.
d. Growth 3 years older than *a*	Relatively few.	
e. Growth 4 years older than *a*	Very few.	
f. Growth 5 years older than *a*	Exceptional.	
g. Growth 6 years older than *a*	None.	
h. Growth 7 years older than *a*	None.	

Thirty-two sample counts, totaling 5,879 cankers from distinct infection waves of five different years in 14 different places, showed the following scale of distribution for the total:

Growth	Percentage of cankers
a	10−
b	53+
c	31+
d	5+
e	0.5−
f	Trace.
g	0
h	0

In the individual counts, *b* and *c* together had consistently 80 to 90 per cent of the cankers, with much the largest percentage (usually more than 50 per cent) on *b*. The percentages on *a* and *d* were relatively small, *a* generally having the larger. The percentages on older growth were almost negligible.

A comparison of the typical distribution pattern with comprehensive sample records of cankers classified according to stage of development and year's growth affected will generally permit an accurate determination of the main infection years and provide a reliable guide to the progress of the rust on pines in any infection area.

Although incipient canker formation from a given year's infection is normally concentrated in the second season after that of infection, it may carry over extensively into the third season. This frequently results in an overlapping in the formation of the cankers and the consequent intermixture of cankers of two successive infection years under one classification in stage of development. Since the rate of development of the individual cankers varies, this intermixture of cankers becomes more common as development advances. The application of the distribution pattern is therefore essential for the accurate determination of infection years.

SOME STORAGE AND TRANSPORTATIONAL DISEASES OF CITRUS FRUITS APPARENTLY DUE TO SUBOXIDATION[1]

By RAY NELSON[2]

Research Associate in Plant Pathology, Michigan Agricultural Experiment Station

INTRODUCTION

Before the investigations of Powell (*15*),[3] Stubenrauch, Ramsey and Tenny (*20*), and Ramsey (*16*) shipments of citrus fruits were subject to the hazard of excessive decay by fungi, especially of the Penicillium type. Rigid application of the principles adduced by these investigators has reduced to a minimum the losses from fungous decays formerly so excessive in transcontinental shipments. The need for further information concerning a group of diseases affecting oranges and grapefruit is frequently emphasized by the conspicuous deterioration that occurs in storage and transportation in the absence of fungous decay. These diseases, unlike the fungous rots, can not be traced to improper handling of the fruit, for they often occur in shipments handled under careful supervision, and in the highest quality brands.

Prior to investigations begun at Michigan State College in 1919, observations during 1917–18 of hundreds of cars of oranges and grapefruit from California, Florida, and Cuba at the Chicago, New Orleans, and Memphis terminals revealed the importance of these diseases. These observations were subsequently extended by numerous inspections of shipments at Detroit from 1919 to 1921. This paper presents the results of experiments on the effect of environmental conditions on the incidence of these blemish diseases, and some tests with chemicals and oiled wrappers in the production and prevention of these diseases.

The major part of this investigation pertains to storage spot or "pox" disease of orange and grapefruit. Some observations and experiments are included on brown spot of navel oranges and brown stain, a disease affecting oranges mainly in cold storage and marine shipments.

EARLIER OBSERVATIONS

That citrus fruits are inherently subject to deterioration under cold-storage conditions was determined by comparatively early investigations. Stubenrauch (*19*) observed that oranges showed external evidence of deterioration in the form of brown spots, pits, and stains after four weeks in cold storage at 32°–42° F., but that no disease developed at 60°–65° in cellar storage.

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[2] The writer is indebted to Dr. E. A. Bessey for a critical reading of the manuscript and translation of the Italian reference.

[3] Reference is made by number (italic) to Literature Cited, p. 712.

Brown spot of Washington navel orange was first described by Smith (*17, p. 47–48*), but Coit (*2*) conducted the first extensive investigations; which, however, failed to disclose the nature and cause of the disease. Later observations by Smith and Smith (*18, p. 1143–1145*) confirmed the previous conclusions concerning the nonparasitic origin of brown spot, but marked no advance in the actual knowledge of the nature and cause of the blemish.

Ramsey (*16*) observed the deterioration of citrus fruits in cold storage and recommended the employment of temperatures considerably above 32° F.; that is, for oranges, 38°–40°; for lemons, about 42°; and for grapefruit, 45°–50°. He noted particularly the development of brown stains and spots on oranges at 32° and a serious spotting of grapefruit at the same temperature.

In a study of the changes occurring in Florida grapefruit in storage, Hawkins and Magness (*9*) found that a pitting of the rind developed at 40° F. or lower. Hawkins (*7*) determined that the amount of pitting could be greatly reduced by storage for one to three weeks at 70°. Later Hawkins and Barger (*8*) recommended treatment with kerosene-stove gas as a partial control of pitting.

Some transportational and storage diseases of vegetables were described by the writer (*13*) and shown to be due to oxygen deficiencies, and some surface blemishes of citrus fruits were similarly classified.

In their investigations of citrus fruit exported from South Africa, Thompson, Putterill, and Hobson (*22*) found it necessary to employ temperatures above 43° F. to prevent brown stain on oranges.

The earlier observations and experiments have shown that brown spot, brown stain, and storage spot are diseases associated with fruit stored at low temperatures or under conditions of poor ventilation. Although these three blemishes are seemingly distinct maladies, it is evident that they are closely related and are to be classified with a group of disorders among which apple scald is the classic example. The breakdown of citrus fruits at low temperatures is not a unique response limited to citrus. Tropical plants in general are apparently more subject to cold injury than those grown in temperatre regions. The spotting of tropical and some other plants when shifted from warm to cold conditions has been noted by several investigators (Molisch (*12*); Harvey (*6*)). A progressive increase in the permeability of the cells of the endocarp of the orange has been shown by Pantanelli (*14*) to occur at low temperatures and indicates the injurious effect of cold on the protoplasm of citrus fruits.

ECONOMIC IMPORTANCE

Since brown spot, storage spot, and brown stain are diseases that do not cause decay, but merely spoil the appearance of the fruit and consequently lower the grade, the actual monetary losses traceable to these blemishes are difficult to estimate. In some seasons the aggregate losses from these diseases to producers and handlers of citrus fruits represent a sum too large to be disregarded. Fruit showing the effects of these blemishes is not only reduced in value out also may influence the general market prices for sound fruit. A few cars of spotted oranges or grapefruit arriving at a terminal may quickly depress prices and impede sales.

As early as 1909, Coit (*2*) observed the serious effects of brown spot on navel oranges in transcontinental shipments. He cited, a depreciation of $307.20 in a car shipped from Riverside to Chicago on December 5, 1909, which on arrival there was diverted to Boston. The development of brown spot or storage spot in this car while moving from Chicago to Boston reduced the market value of the fruit 50 per cent. The total elapsed time from picking to sale was three weeks.

Just after Thanksgiving in 1920, the writer saw two cars of Florida grapefruit and 500 cases of navel oranges sold at the Detroit terminal for slightly more than transportation charges. All of this fruit was affected with storage spot. (Pl. 1.) Fruit arriving through December and into January of the same season was severely spotted but later little or no disease was apparent until April, when some shipments were again badly affected. Similar examples might be cited for other terminals and seasons.

Oranges affected with brown spot and oranges and grapefruit showing the effect of storage spot have been observed each year on the Lansing and Detroit markets. Usually fruit shipped during November and December is most seriously affected, but sometimes summer and late-season shipments are badly spotted. (Pls. 1, C; 2, D.) Although spotted fruit may be observed on the markets each year, seasonal variations in the incidence and severity of the blemishes influence the conspicuousness and consequent decline in the value of the fruit.

Fawcett and Lee (*3*) reported much cold-storage spotting in eastern markets during July and August 1924, on grapefruit held in cold storage for 5 to 12 weeks. Bratley [4] observed that California Valencia oranges arriving on the New York market in 1931 were more severely affected with spots and pitting than during previous years.

SYMPTOMS

STORAGE SPOT OR POX

The first symptom of storage spot on citrus fruits is the occurrence of slightly sunken spots of variable size over the surface of the fruit. (Pl. 3, B.) Inspection shows, in most cases, that the cells surrounding the oil glands are the first to be involved, these cells collapsing and leaving the oil glands standing out in relief above the sunken tissue. (Pls. 4, E; 3, B.) The lesion may increase in size for 24 hours or more, but usually its limits are determined by the initial collapse of the cells after exposure of the fruit to conditions favorable for the production of the disease. The spots may be so small that the area of collapsed tissue embraces only the cells surrounding a single oil vesicle, or the lesions may be several centimeters in diameter. (Pl. 1, B.) Often the diseased areas exceed one-half the surface of the fruit. (Pl. 1, B and C.)

The lesions at first are typically angular (pls. 1, C; 3, B), but eventually they assume a more or less circular outline (pl. 1, A and B). Secondary extension by fungi of a newly formed lesion seldom occurs, but old spots are sometimes invaded. As the lesions age the oil

[4] BRATLEY, C. O. NEW YORK MARKET PATHOLOGY NOTES. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 15:133. 1931. [Mimeographed.]

glands also collapse through desiccation of the adjacent cells, forming a depressed area with a sharply defined, darker border. The oil vesicles, when fully dried out, are sometimes more deeply sunken than the adjacent parenchyma cells. (Pl. 1, B.) In exceptional cases the cells over the oil glands are first affected and become depressed below the surface level of the fruit before the surrounding cells collapse.

The affected tissue is colorless at first but gradually changes to various shades of brown. The most common color of the grapefruit lesion is russet vinaceous.[5] On oranges the spots show a greater variety of color gradations, usually ranging from Natal brown to several vinaceous tints. The lesions are darker in the early-shipped fruit, and the color becomes less intense as the season advances until on spring and summer shipments of grapefruit and late shipments of navel oranges the spots may be almost colorless. (Pl. 1, C.) The lesion is always more deeply colored at the border, but this may not be apparent in the early stages of development. The darker color of the border is usually some shade of purple.

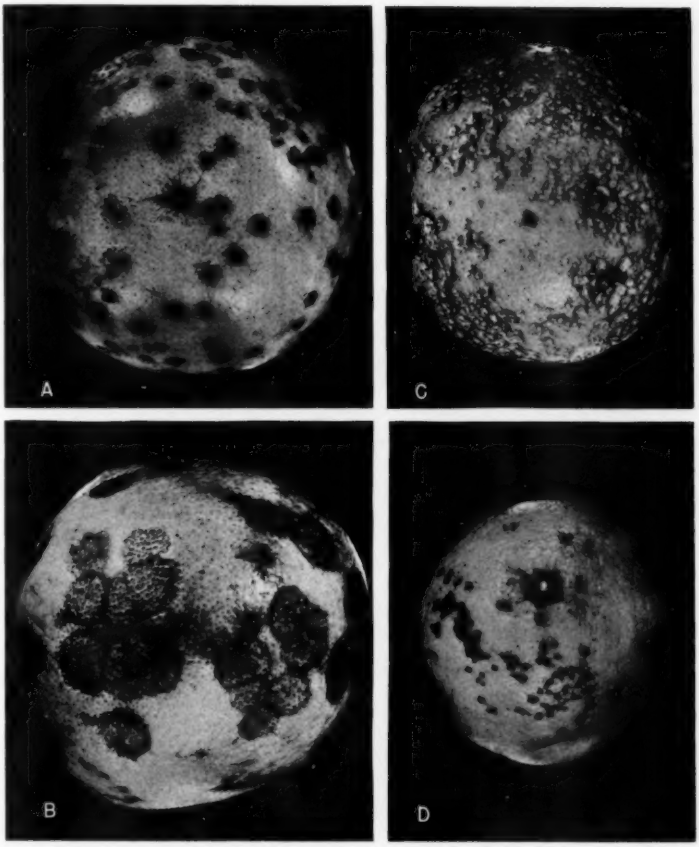
When examined in refrigerator cars, oranges and grapefruit may show no evidence of pitting, but if delay has occurred in transit or the fruit has been left in the car for some time after arrival, spotting may become evident. In cars frequently diverted breakdown may increase greatly in amount and severity and the fruit be seriously reduced in value before it reaches the market. Fruit held four to eight weeks at temperatures close to 32° F. develops characteristic lesions while still in storage. Unlike apple scald, which does not complete its development until the fruit is removed to a warm place, storage spot may become evident in two to four weeks, but the color typical of the lesions does not always develop until the fruit is removed to a higher temperature.

Histologically the changes that occur in the affected areas have their origin in the collapse of the parenchyma cells that surround the oil vesicles. The anatomical features of the normal peel are shown in Plate 4, A, and the general appearance of sections through storage-spot lesions on grapefruit are illustrated in Plate 4, B–D. The initial collapse of the active parenchyma cells around the oil glands is followed by the gradual desiccation of the affected cells and the formation of a depressed lesion. The edges of the lesions are bordered by a zone of deeper-colored cells also in a state of collapse. (Pl. 4 C and D.) In these cells there is a pigment that is absent from the other portions or is present in smaller quantities.

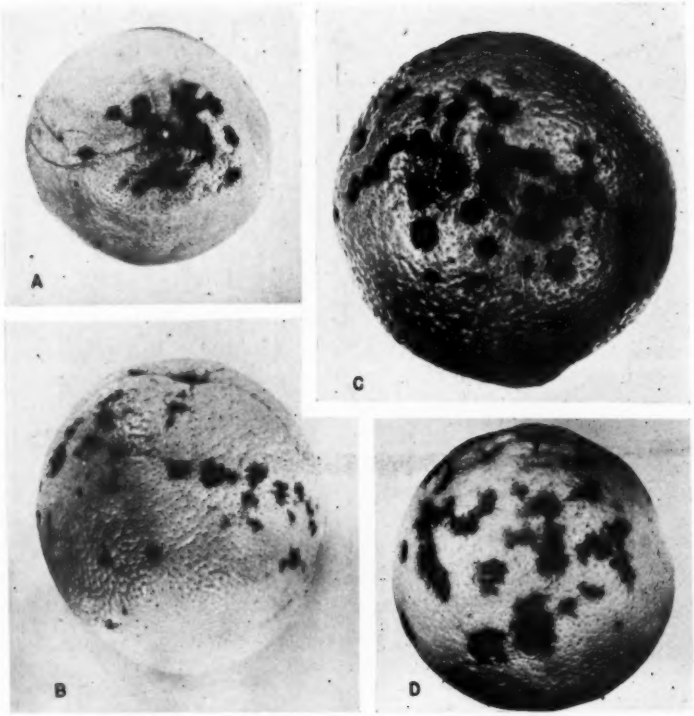
BROWN SPOT

In general, brown-spot lesions on navel oranges do not, in the final stages, differ greatly from those of storage spot. Usually the lesions of brown spot are shallower, more uniformly circular in outline, deeper in color, and limited to thin-skinned navel oranges. Brown-spot and storage-spot lesions have been produced on the same orange under the same experimental conditions. (Pl. 3, C.) Unquestionably, storage spot has frequently been confused with brown spot, and the greater susceptibility of those navels with smooth thin skins to both of these diseases has made distinction difficult. In general, the

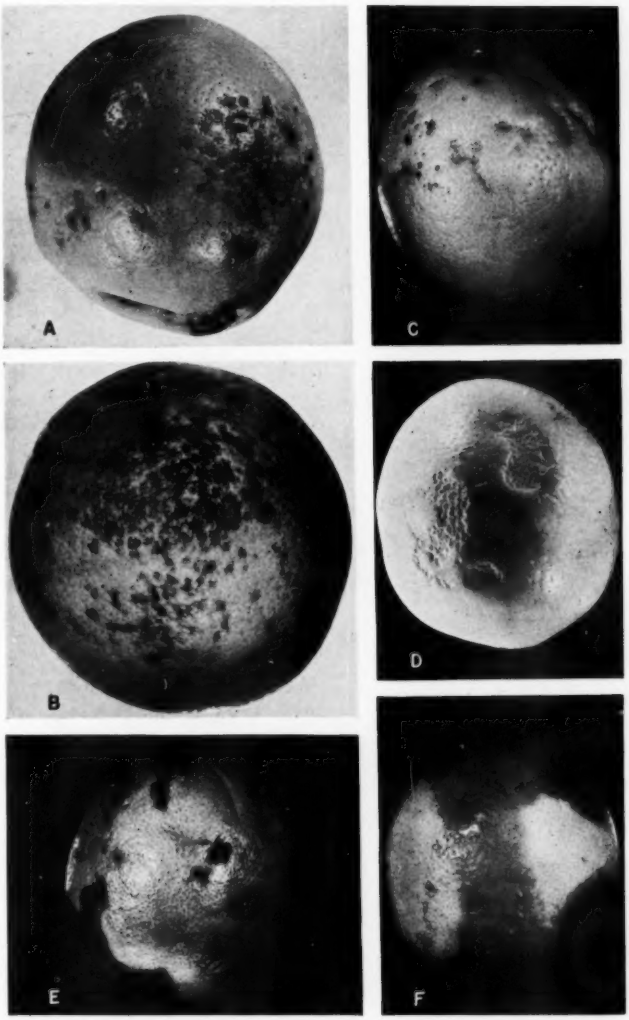
[5] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.



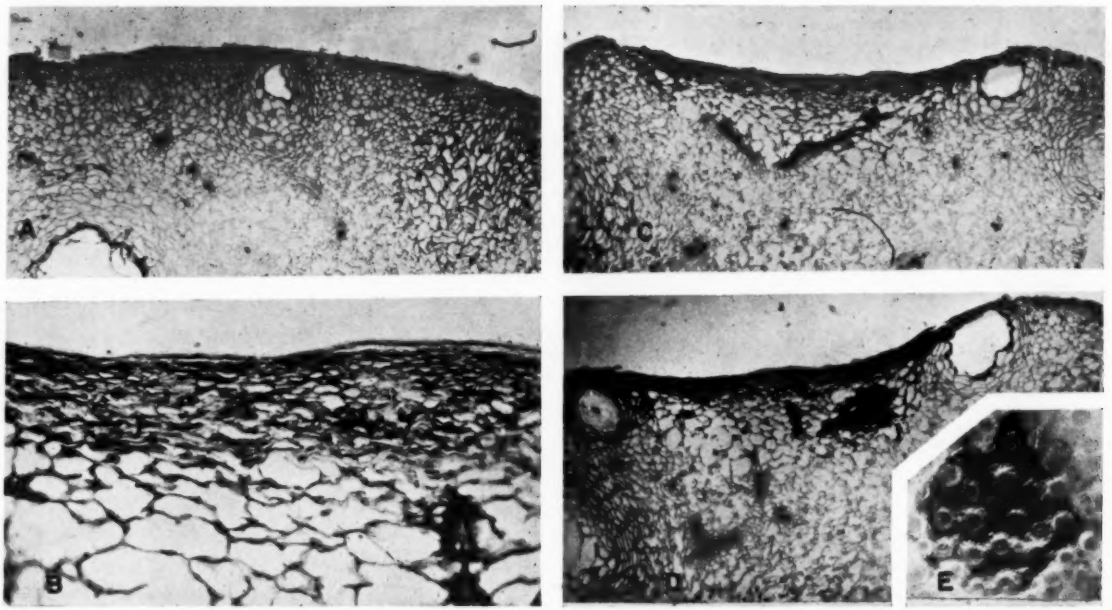
A, Florida grapefruit affected with storage spot which developed in transit. Collected March 30, 1920. B, Florida grapefruit showing a severe form of storage spot which developed in transit. Collected at Detroit terminal December 12, 1920. C, Florida grapefruit affected with storage spot which developed in cold storage. Collected July 1931. The borders of the lesions are not so dark in color as they usually are on fruit affected earlier in the season. D, Texas-grown, Marsh Seedless grapefruit affected with storage spot. Collected March 2, 1930.



A, Washington navel orange affected with brown spot at the stem end. Collected at Detroit, 1919. B, Washington navel orange affected with brown spot. Collected at Detroit, March 1920. C, Washington navel orange affected with storage spot. Specimen from a late shipment, June 1, 1926. Sometimes very ripe and old oranges and grapefruit are severely affected with this disease but usually immature fruit is more susceptible. D, Valencia orange showing the effect of storage spot. November 2, 1928.



A, Storage-spot lesions produced on Washington navel orange by storage in nitrogen for 13 days at room temperature. Thirty-minute aerations with nitrogen were given on 6 successive days. April 2, 1920. B, Washington navel orange held in open basket in cold storage for 109 days at a temperature of 34°–36° F. The fruit was green skinned when stored but gradually assumed a yellow color in storage and at the time of removal, April 23, 1929, was normal in appearance. Typical lesions of storage spot developed after 2 days at room temperature. C, Washington navel orange stored in nitrogen at room temperature for 7 days. Brown-spot and storage-spot lesions developed after 3 days in air. D, Breakdown produced on Washington navel orange by storing in oxygen-free air for 4 days. The oxygen was removed from the storage jar with chemicals. March 1920. E, Lesions resembling those of brown spot produced on Washington navel orange by a short exposure to the vapor of acetaldehyde. January, 1932. F, Brown stain on Washington navel orange produced by exposing the fruit to the dilute vapor of citral. Similar but less pronounced injury follows exposure to vapors of various acetates.



A, Section through the normal peel tissue of grapefruit. × 25. B, Section through a storage-spot lesion on grapefruit. The cells have collapsed and form a compressed layer of disorganized tissue. × 80. C, D, Sections through storage-spot lesions on grapefruit. The collapse of the parenchyma cells and the darker colored border of the lesion is evident. × 25. E, Surface view of a storage-spot lesion on grapefruit in an early stage of development. The cells surrounding the oil vesicles are affected, but no collapse of the oil glands has yet occurred. × 8.

storage-spot disease is distinguished from brown spot by the more angular outline, deeper penetration into the peel, and lighter color of the lesions. Since, however, the color changes depend on the season and other variable factors, no distinctive color tones can be assigned to the lesions of either disease.

The brown-spot lesions, like those of storage spot, occur most frequently at the stem end of the fruit. This is particularly true of fruit shipped after the period of greatest susceptibility. Sometimes a few small lesions at the stem end represent the extreme development of the disease in a season. In other years the disease develops much more seriously and the fruit is badly disfigured by numerous spots over the surface.

As pointed out by Coit (*2*), the color of the lesions varies with the season. In some years the spots are dark brown to black and in others they are much lighter. The writer has found that as the season advances the spots become lighter in color, but occasionally fruit is observed in January or later on which the spots are almost black at the stem end. (Pl. 2, A.) The deeper color of the lesions of brown spot on fruit grown in the interior valleys and the lighter color on fruit from the coastal regions, reveals the extreme variability that may be found in the color of the spots on oranges after they reach the markets.

BROWN STAIN

Brown stain is a surface blemish of oranges occurring on fruit in storage, occasionally being evident also in shipments that have been long delayed. It is an important disease at low temperatures in marine shipments of oranges which are in transit for more than three weeks. It has been troublesome on export fruit from South Africa and has been observed on oranges held in storage for several weeks at temperatures below 40° F. The disease is manifest as a brown staining of the skin or flavedo, and in the beginning is unaccompanied by any depression of the surface cells. The color of the affected area is usually dark brown, closely approximating the Natal brown or cinnamon brown of the brown-spot lesions. This blemish is probably closely related to adustiosis or red-blotch disease of lemon and shows many similarities to apple scald. In the later stages, after the fruit has been exposed to drying for some time, the affected tissue is leathery and shriveled and the oil glands are slightly sunken below the level of the surrounding cells. Except in severe cases, there is no marked depression or pitting such as characterizes the lesions of brown spot and storage spot.

EXPERIMENTS

EFFECT OF OXYGEN

The experimental work on the artificial production of the diseases that have been described was begun in 1919. The first experiments were planned in conjunction with work being done on some related diseases of cabbage and other vegetables. The conditions under which the citrus diseases developed in transit and storage and the success that had been attained in reproducing the vegetable diseases in the laboratory, led to the inclusion of some experiments with oranges and grapefruit under conditions which had been effective in producing black-leaf speck of cabbage.

The first experiments consisted in storing oranges and grapefruit in large, air-tight jars with suitable connections so that aerations could be given with nitrogen or other gases. Breakdown as sunken colorless lesions, later turning brown developed on both grapefruit and oranges stored for four days in nitrogen with the gas content of the storage jars changed every 24 hours. With some variations the first experiments were repeated a number of times with similar results. In cases in which the oxygen was removed from the storage jars with pyrogallic acid and sodium hydroxide, fruits left in the jars for seven days or more developed spots in the peel similar to those observed on fruits in the market. On oranges the lesions were very similar to those of brown spot and storage spot, while on grapefruit they resembled the spots on blemished fruit from refrigerator cars and cold storage.

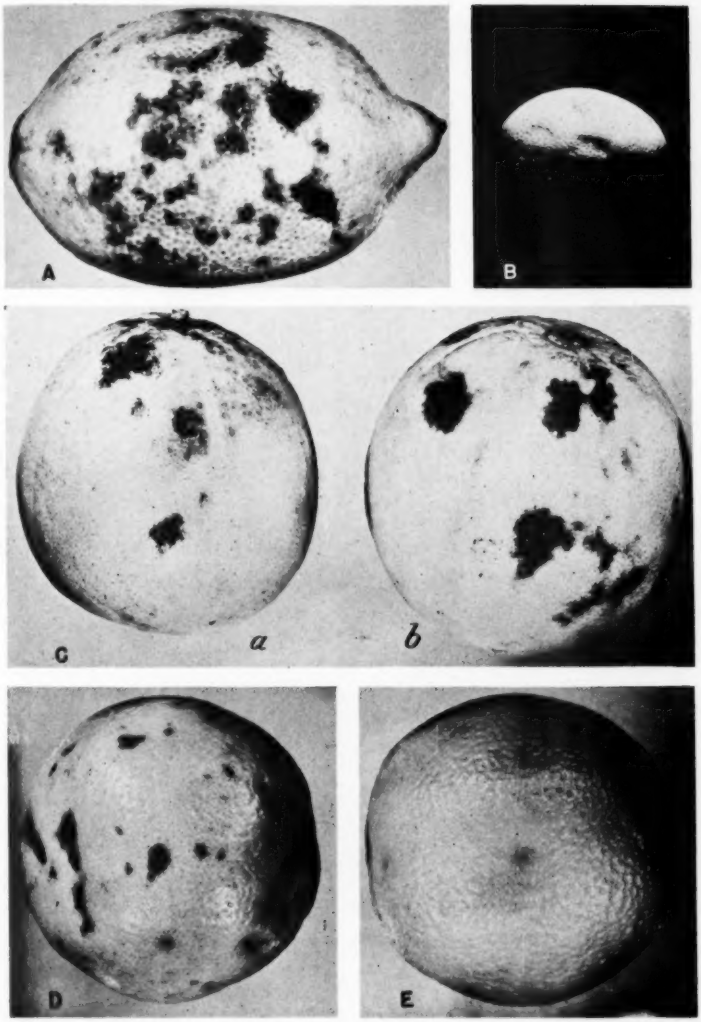
Experiments devised to determine the conditions under which breakdown could be produced were resumed in December, 1920. On December 8, fully colored grapefruit, Valencia oranges, California navel oranges, and lemons were stored in large, closed glass chambers of 12-liter capacity. Outlets to the storage jars were connected with tanks of nitrogen and the air in the jars was displaced with this gas. Aerations of 30 minutes duration were given daily with nitrogen, thus effecting a complete change of the gas in the jar. The oranges were stored at room temperature and also in the refrigerator at approximately 50° F. After seven days, all of the jars were opened and the fruit was held at room temperature for three days. The results of this test, which are similar to those obtained in other tests are given in Table 1.

TABLE 1.—*Effect on citrus fruits of storage in nitrogen for seven days at different temperatures*

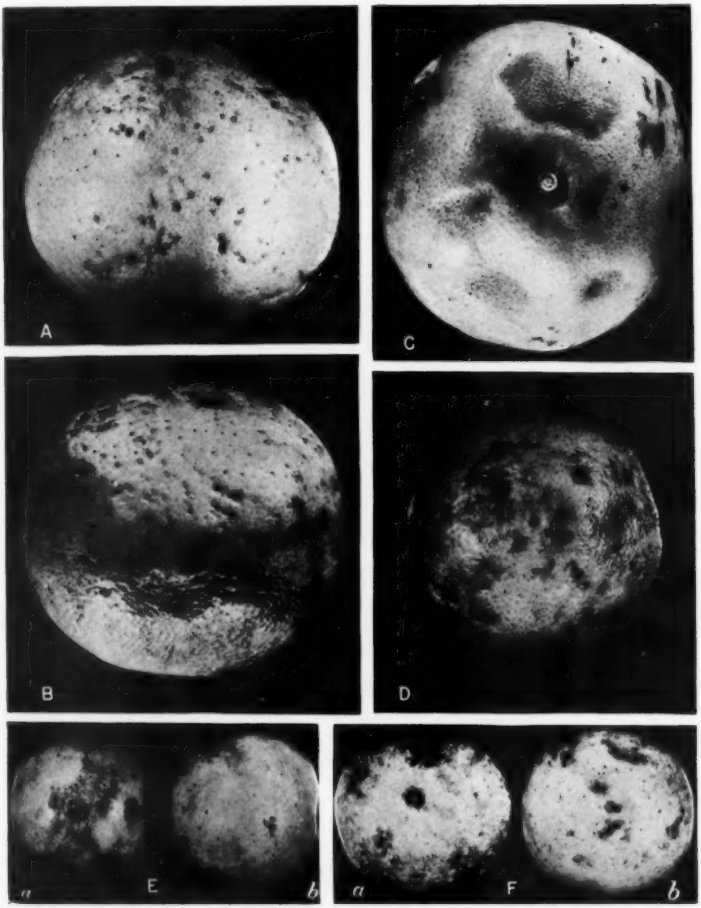
[Fruit kept in air-tight jars; nitrogen changed daily]

Lot No. and kind of fruit	Temperature	Results
	°F.	
1, oranges	70	Slight pitting evident on fruits before removal from jars; when exposed to air all fruits developed numerous sunken spots which gradually became Natal-brown in color.
2, oranges	50	Fruits developed sunken brown spots on exposure to air; spots 1–2 cm in diameter, irregular border, Natal-brown color.
3, grapefruit	70	Pitting visible on the fruits after 3 days in jars; very numerous after 7 days; small pits involving only small area around oil glands; border of the spots irregular; spots more numerous at stem end; hydrangea-red color after 3 days at room temperature.
4, lemons	70	All fruits developed deeply depressed spots in the peel; 1–2 cm in diameter, colorless at first, pale vinaceous after 3 days.

When the oranges were removed from the storage jars only a few lesions were visible in the peel, but after exposure to air, pitted areas developed over the surface. The lesions were colorless at first but within 24 hours the affected areas were cinnamon brown. (Pl. 5, C, *a*.) The spots were sharply sunken and the oil glands had collapsed also. On some fruits, brown scalded areas were evident. The scalded tissue gradually dried out and the affected areas finally appeared on the peel as slightly depressed, Natal brown spots. Small sunken spots were visible on the grapefruit after three days in the storage jar, and after seven days the number and size of the lesions had greatly in-



A, Lesions of the storage-spot disease on lemon collected December 3, 1923. B, Breakdown in the form of sunken lesions produced on lemons by storage in nitrogen atmosphere for 5 days at room temperature. C, Brown spot of Washington navel orange (*b*) on fruit collected January 4, 1920, and similar lesions produced on thin-skinned navel orange (*a*) by storing in nitrogen for 7 days. The gas in the storage jar was changed daily by a 30-minute aeration with pure nitrogen. D, Brown-spot lesions occurring on Washington navel orange after storage in nitrogen for 5 days. December 4, 1920. E, Washington navel orange from the check of the same experiment (D) stored in air-tight jar for 5 days and given a daily aeration of 30 minutes with air of normal composition.



A, Storage-spot lesions developed on green-skinned Florida grapefruit after storage for 7 days at 40° F. B, Storage-spot lesions on green-skinned Florida grapefruit stored 10 days in air constantly circulated but not renewed. (See Table 3.) The lesions developed before and after removal from the storage jar. Experiment begun February 5, 1921. C, Large lesions on green-skinned Florida grapefruit stored in air-tight jar for 10 days. Aerations of 30 minutes with nitrogen were given each day. D, Sunken lesions, which gradually developed a vinaceous color, produced on grapefruit by exposure to the dilute vapor of acetaldehyde and acetic acid. E, Texas grapefruit that has been wrapped in commercial (*a*) and oiled (*b*) wrappers and stored for 6 weeks at 34°–36° F. Storage spot is severe on *a*, but *b* is almost free from spotting. F, Texas grapefruit that had been wrapped in commercial (*a*) and oiled (*b*) wrappers and stored for 7 weeks at 34°–36° F. The fruit from both the oiled and commercial wrappers spotted badly. The same results were obtained with fruits from the same shipment stored under identical conditions for 10 weeks.

creased. The spots were formed by the collapse of the cells surrounding the oil glands. Later the glands also were involved. The lesions were not colored when the fruit was taken from the jars but 48 hours later they were hydrangea red. The spots varied from 2 mm to 4 cm in diameter, and were mostly circular in outline.

The lemons were affected with deeply sunken, colorless lesions before they were removed from the storage jar. When the jar was opened a strong odor of lemon oil was detected. The spots in the peel were 1 to 2 cm in diameter and resembled the lesions of the peteca disease. (Pl. 5, B.) They were colorless when the fruits were taken from the jars but after 3 days the affected tissue was pale vinaceous.

This experiment is typical of others in which early-season ripe fruit was used. In practically all cases, breakdown in the form of sunken lesions of variable size has followed storage of the fruit in nitrogen for 5 to 10 days. Oranges or grapefruit that are still green in color are more susceptible to injury than yellow fruit. The reaction of green grapefruit to similar storage conditions was tested with some market fruit shipped in October, 1921, from the Manatee River district of Florida. The fruit was stored on October 21 in large glass jars. A stream of nitrogen was blown constantly over the fruits in some of the jars; in others the gas was not changed. The result was the same, breakdown occurring in the form of sunken colorless spots in the peel after three days of storage at room temperature. This represents about the minimum time necessary to produce breakdown by storing fruit in the most susceptible condition in nitrogen. A much longer period of storage is necessary to produce spotting on yellow fruit, and in some cases breakdown has not occurred after storage in pure nitrogen for seven days.

In the preliminary tests with oranges, similar results had followed storage in nitrogen and in air from which the oxygen had been absorbed. The results of a typical experiment with navel oranges stored in nitrogen are shown in Table 2.

TABLE 2.—*Effect of various storage conditions in producing surface breakdown of navel oranges in tests at room temperatures, March 25, 1920*

Lot No.	Method of storing	Storage period	Results
		Days	
1	Oxygen absorbed with pyrogallic acid and KOH; aerated 30 minutes daily with nitrogen.	4	All oranges developed sunken, colorless spots on exposure to air; spots numerous, variable in size; pecan-brown color after 3 days.
2	Same as 1, but aerated 1-hour daily with nitrogen.	7	Sunken, colorless spots visible in the peel after 5 days; when removed from the jars all oranges developed numerous sunken colorless spots over the surface; orange-cinnamon color after 3 days.
3	Oxygen absorbed with pyrogallic acid and KOH; no aeration with nitrogen.	4	When removed from the storage jars, all oranges developed numerous large sunken lesions, formed at first by the collapse of the cells around the oil glands; pecan-brown color after 3 days.
4	Stored in pure nitrogen; gas not changed..	4	Lesions similar to those on the fruits from jar 3; 1–2 cm in diameter; pecan-brown color.
5	Stored in pure nitrogen; aerated 1 hour daily with nitrogen.	13	Fruits severely spotted with large, sunken lesions similar to those on fruits in jars 3 and 4.
6	Stored in air-tight jar; aerated 1 hour daily with air of normal composition.	13	All fruits sound.
7	Wrapped with commercial wrappers and held in open basket.	13	Do.

The reaction of oranges to storage in nitrogen or in air from which the oxygen had been absorbed was not different from that of grapefruit under similar conditions. In the jars aerated for one hour daily with nitrogen, breakdown was as severe as in others in which the gas was not changed. Tests with navel oranges in January, 1932, gave similar results. Thin-skinned navel oranges were stored in two large glass jars and the air in one was displaced with nitrogen. The fruit in the second jar was exposed to a constant stream of nitrogen, which resulted in a complete change of the gas in the jar every hour. At the end of seven days the oranges were removed from the two jars and held at room temperature for three days. No breakdown was visible at the termination of the storage period on any of the fruits which had been exposed to a constant stream of nitrogen, but typical pox lesions developed within three days. Of the fruits from the jar in which the nitrogen was not changed, characteristic brown-spot lesions were evident on one when the jar was opened. At the end of three days typical storage-spot lesions had appeared on all the fruits.

The results of this and similar experiments indicate that breakdown occurs when oranges are stored for a short time in air freed of oxygen. The period of exposure required to cause injury depends on the condition of the fruit. Some tests have been conducted with green-skinned navel oranges shipped directly to the writer from the grove in California. Fruit of this kind breaks down much more rapidly under the storage conditions that have been described than ripe oranges. These results are in accord with many observations which have shown that fruit in a slightly green condition is much more susceptible to spotting in transit than ripe fruit.

EFFECT OF AIR MOVEMENT

In the experiment with oranges and grapefruit stored in nitrogen, breakdown was not prevented by blowing a constant stream of gas over the fruit. Brooks, Cooley, and Fisher (*1*) have demonstrated the beneficial effect of air movement in dissipating the volatile products of respiration which injure the superficial layers of the cells of apples in cold storage. Since apple scald has been well controlled by aeration, and also by the use of wrappers which absorb these toxic substances, the importance of determining whether or not the citrus breakdown was due to similar causes was early recognized. Some experiments on air movement and the use of wrappers impregnated with absorbents effective in the control of apple scald were devised to determine, if possible, the nature of the citrus diseases.

The first experiment on the effect of air movement was carried out in September, 1921, with green Isle of Pines grapefruit. The fruit was stored in 14-liter jars, and an apparatus illustrated in a previous publication (*13*) was used to circulate the air in the storage chamber. The experiment was conducted at room temperature but, owing to the warming of the air by the heat from the motor-driven fan, the temperature of the circulating air was approximately 77° F. The fruit was left in one jar for 7 days and in the second for 13 days. When removed from the first jar at the end of seven days the fruits were perfectly normal in appearance, yellow, and free from blemishes. After three to five days, small, sunken spots, beginning as a collapse of the cells surrounding the oil vessicles, appeared on all the fruits.

The spots were 1 to 5 mm in diameter, and although colorless when first exposed to the air, they became cinnamon brown within three days. There was no off-taste or impairment of the eating qualities of the fruit as a result of the storage conditions.

The second jar was opened at the end of 13 days; at this time some of the fruits were already spotted but 3 days later the number and size of the lesions had greatly increased. This fruit also compared favorably in flavor with the check fruit ripened in the air of the laboratory. The development of the spots and color changes were similar to those on the grapefruit stored for only seven days.

A second experiment of a similar nature was started on February 5, 1921, with slightly green grapefruit. The air was circulated around the fruit but not renewed. In a second jar the fruit was stored in nitrogen and the gas changed every 24 hours by a 30-minute aeration. The results of this experiment are shown in Table 3.

TABLE 3.—*Effect of composition and movement of storage air on pitting of grapefruit in tests at room temperature during a 10-day storage period*

Lot No.	Method of storing	Results
1	Air stirred constantly but not renewed	Pitting in the peel evident after 7 days. After removal from jars all fruits developed few to many spots; spots hydrangea red after 3 days; fruit normal in flavor and color.
2	Stored in nitrogen and aerated 30 minutes daily with this gas.	Fruits severely disfigured by sunken areas in the peel; evident after 5 days but developed more severely after the fruit was removed from the storage jar; hydrangea red after 3 days.
3	In open basket	All fruits sound, slightly shriveled, yellow.

Lesions very typical of storage spot appeared on the fruits from the jars in which the air was circulated but not renewed. The spots were generally small, but on some fruits large areas were involved by the collapse of the cells surrounding the oil vesicles. (Pl. 6, B.) The lesions on the fruits stored in nitrogen were much larger, consisting of sunken areas which included the oil glands as well as the adjacent cells. These lesions were visible on the fruits before they were removed from the storage jar but were colorless until exposure to the air of the laboratory caused color changes similar to those previously described.

Although in these experiments a continuous circulation of the air in the storage jars did not prevent the development of breakdown, it is not to be concluded that under conditions more nearly like those prevailing in storage and transportation, air movement would not have a beneficial effect. In these jars the temperature was much higher and the relative volume of fruit per unit volume of air much in excess of that to which citrus fruits are usually exposed in storage and transportation.

EFFECT OF TEMPERATURE

In the preceding experiments all of the tests except one were carried out at room temperature. To determine the effects of low temperature on grapefruit, a case of green Florida fruit was purchased in November, 1925, and stored at various temperatures. In general, all the fruit used was green-mature but some variation in color was unavoidable. The storage conditions and temperatures employed are indicated in Table 4.

TABLE 4.—*Effect of temperature on the development of storage spot or pox on green-skinned grapefruit stored by different methods for varying periods*

Lot No.	Method of storing	Temperature	Storage period	Results
		° *F.*	*Days*	
1	In wooden box with closely fitting lid.	31–33	22	A few fruits pitted at time of removal from storage; within 2 days after removal from storage every fruit affected with typical storage spot.
2	In 12-liter, air-tight glass jar	40	17	All fruits affected with typical storage-spot disease within 2 days after removal from storage.
3	In open basket	31–33	22	Fruits badly spotted within 24 hours after removal from storage.
4	do	40	17	Do.
5	do	70	17	All fruits sound.
6	do	50	42	Do.

As Table 4 shows, the fruits stored in open baskets or in closed jars spotted badly at the lower temperature. There was no deficiency of oxygen in the air of the storage rooms at any of the temperatures employed. In the 31°–33° F. room absence of other living material precluded the possibility of the oxygen content of the air being reduced. In addition, the doors of this room were opened at frequent intervals during the day for the removal of dairy products. Despite the abundant supply of oxygen in the air, the fruits in open baskets spotted as badly as those in the closed containers. Since the grapefruit in the open baskets were not wrapped, free movement of air around each fruit was not prevented. At both 50° and 70° no spotting developed on any of the fruits.

Although the results of the experiments on air movement, temperature, and aeration with nitrogen indicate that spotting of grapefruit is not produced by any volatile substance acting at the surface of the fruit, there is not excluded the possibility that a slow, cumulative adsorption of such substances at low temperatures may cause this type of injury. Whatever the cause of the injury may be, the tolerance of green fruit to cold is very low compared to that of apples. Spotting occurred in one case on green fruits after only seven days storage at 40° F. (Pl. 6, A.)

INFLUENCE OF MATURITY

In some of the experiments described it was noted that green fruit was much more susceptible to injury at both high and low temperatures than ripe, yellow fruit. Coit (*2*) noted the greater susceptibility of early picked oranges to brown spot, and Hawkins and Magness (*9*) observed that grapefruit were most susceptible to breakdown in cold storage immediately after picking. To compare the resistance of green and ripe fruit some tests were made in November, 1925, with Florida grapefruit. Two cases of grapefruit from the same shipment were obtained. One lot was green in color and the other had the normal yellow color of ripe fruit. Uniformly green and fully colored fruits were selected and stored as indicated in Table 5.

TABLE 5.—*Influence of maturity on the susceptibility of grapefruit to breakdown at various temperatures when stored for different periods of time*

Color of fruit	Method of storing	Temperature	Storage period	Results
		°F.	Days	
Green	Tightly closed wooden box	31–33	22	All fruits spotted after 48 hours at room temperature; an occasional fruit was already spotted when removed from storage.
Do	Air-tight glass jar	38–42	17	Spotted after 3 days at room temperature.
Do	Open basket	31–33	22	All fruits spotted within 24 hours at room temperature.
Do	do	38–42	17	Spotted after 2 days at room temperature.
Do	do	70	17	All fruits sound, yellow in color, and slightly shriveled.
Yellow	do	31–33	28	All fruits sound.
Do	do	38–42	17	Do.
Do	do	31–33	42	Do.
Do	do	48–52	42	Do.

The maximum period of storage for the green fruit was 22 days, but one lot of the yellow fruit was left for 42 days at the lowest temperature. In all cases green fruit stored at 31°–33° and 38°–42° F. became spotted before or shortly after removal from storage. At the lower temperature some of the fruit spotted while still in storage, but most of the spotting occurred after exposure to the warm air of the laboratory. At the end of 42 days, the yellow fruit stored at 31°–33° was still sound and remained so after exposure to warm conditions.

In experiments with other lots of grapefruit, results similar to these were obtained. Fruit that is mature and fully colored is comparatively resistant to storage spot. This is more especially true of midseason shipments. Fruit that is fairly well colored but picked early in the season seems to be more susceptible than similarly colored fruit picked later. According to legal standards, grapefruit and oranges with green skins may be shipped if they contain sufficient sugar and the acid content is low enough. In these shipments, which often arrive at destination in a green condition, breakdown is frequently very severe. Both grapefruit and oranges of midseason and late shipments are ordinarily fully colored and mature and usually are not seriously affected with storage spot.

Some exceptions have been observed where storage spot has developed severely on ripe and very old fruit. Similar exceptions to the rule that immature apples are more susceptible to apple scald have been noted by Brooks, Cooley, and Fisher (*1*).

EFFECT OF OILED AND WAXED WRAPPERS

The beneficial effect of oiled wrappers in the control of scald on apples in cold storage suggested the possibility that the use of these might prevent the spotting of citrus fruits in transportation and storage. The first wrappers tested were prepared by treating the ordinary commercial wrappers with oilve oil, beeswax, and cocoa butter according to the method used by Brooks, Cooley, and Fisher (*1*). In a test conducted in 1920, grapefruit and oranges wrapped in the prepared wrappers and stored in nitrogen at room temperature for 12 days spotted badly when exposed to the air.

In 1924 the writer obtained some commercially prepared oiled wrappers through the courtesy of D. F. Fisher of Wenatchee, Wash. These wrappers carried about 12 per cent of mineral oil and were effective in preventing apple scald. An early shipment of Indian River grapefruit obtained October 14, 1924, was used for the first test. The fruit was slightly green and had a smooth, fine-textured skin. Since no cold-storage facilities were available, the fruit was stored only at room and ice-box temperatures. The results of this test are shown in Table 6.

TABLE 6.—*Effect of oiled wrappers on the spotting of grapefruit, when stored for different periods of time*

Lot No.	Method of storing	Temperature	Storage period	Results
		° *F.*	*Days*	
1	Wrapped in oiled wrappers; stored in air-tight jars.	70	10	All fruits spotted 2 days after exposure to air at room temperature; spots small and few in number.
2	Wrapped in commercial wrappers; stored as in 1.	70	7	All fruits slightly spotted after 3 days at room temperature.
3	Unwrapped; in open basket.....	70	10	All fruits sound.
4	Wrapped in oiled wrappers; in air-tight jar.	50	8	Do.
5	Wrapped in oiled wrappers; fruit stored in open baskets.	50	10	Do.

At room temperature the oiled wrappers were ineffective in preventing spotting of the fruits in closed jars. Analyses made every 24 hours of the gas content of the jars kept at room temperature showed that the oxygen was rapidly depleted. After 64 hours only 1.5 per cent oxygen remained in the jars, and after 108 hours no test for this gas could be obtained by the method used (Ganong (*5*)). At the lower temperature no breakdown developed on any of the fruits, either in the air-tight jars or in open baskets. The storage period was too short at this temperature to cause breakdown, although in some earlier tests, green-skinned fruits had become spotted in seven days at temperatures slightly above 32° F.

Florida grapefruit wrapped in commercial and oiled wrappers and stored for six weeks, beginning January 13, 1932, at temperatures of 34°–38° F. became slightly spotted after removal from storage. None of the fruits in either kind of wrapper was seriously spotted. On March 1, 1932, a second experiment with the oiled wrappers was started in which mature, fully colored, Texas-grown Marsh Seedless grapefruit were used. One lot was stored at 34°–36° for 6 weeks; a second lot was stored at 34°–38° for 7 weeks, and a third lot was stored at 34°–38° F. for 10 weeks. Fruits wrapped with the ordinary unoiled commercial wrappers were stored under the same conditions. The oiled wrappers reduced the amount of spotting on the fruits stored for 6 weeks at 34°–36° (pl. 6, E), but those stored for 7 weeks at 34°–38°F. spotted as badly in the oiled as in the commercial wrappers (pl. 6. F). The oiled wrappers had no apparent beneficial effect on the fruits held for 10 weeks at the same temperature.

In December, 1925, navel oranges wrapped in the commercial unoiled wrappers and the same brand of oiled wrappers that were used in the grapefruit experiments were stored for 45 days at 34°–38° F. The first spotted fruits were found in the oiled wrappers after 34 days

of storage; after 45 days all the fruits were removed from storage, unwrapped, and held for 3 days at room temperature. None of the oranges had spotted badly, but those from the oiled wrappers were in no better condition than those from the commercial wrappers.

In a similar experiment with navel oranges wrapped in both commercial unoiled and oiled wrappers and stored January 13, 1932, at 34°–38° F. for 47 days slight spotting developed on all the fruits from both the oiled and the unoiled wrappers. There was slightly more spotting on the fruits from the unoiled wrappers, but the difference did not appear to be significant.

The favorable influence of the oiled wrappers in some of the grapefruit experiments warrants further trials of this method, with wrappers of higher oil content and fruit freshly picked from the trees. It is possible that these wrappers might delay the appearance of the disease in early shipments of grapefruit and oranges. Friend and Bach (*4*) have recently reported beneficial results from waxed wrappers on Texas grapefruit in cold storage.

EFFECT OF CHEMICALS

The discovery by Brooks and his associates that certain volatile emanations of apples are the probable cause of scald in storage marked a milestone of progress in the search for methods of controlling this very serious disease. The results obtained in the experiments conducted to produce scald under laboratory conditions influenced the writer to test various chemicals for their effect on citrus fruits. These experiments, like the preceding, were conducted with fruit that was not especially favorable for experimentation. It is believed that the results would have been more decisive had the fruit been in the most susceptible condition.

Recent investigations by Klotz (*10*), of red blotch, and peteca of lemon, diseases that develop in the curing house where ventilation is poor, have shown that these blemishes are probably caused by the action of toxic substances produced in the respiratory processes of the fruit. Touching the fruits with a number of esters or injecting small amounts of citrus oils under the flavedo produced lesions typical of these two diseases.

Various substances besides a number of aldehydes, esters, and alcohols that are concerned in the chemistry of citrus, were used in attempts to simulate the effects of brown stain, brown spot, and storage spot on oranges and grapefruit. The chemicals tested included acetaldehyde, acetic acid, citral, citronellal, geraniol, methyl anthranilate, linalool, *d*-limonene, ethyl acetate, methyl acetate, amyl acetate, and octyl acetate. The method used was to expose the fruit in closed vessels to the chemical in a vaporized condition. One to two milliliters of the chemical to be tested was poured in the bottom of an 8 to 12 liter desiccator and the fruit was placed on supports a few inches above the concentated material. The desiccators were kept at room temperature or in the refrigerator at 40° F. The period of exposure varied from 2 to 24 hours, depending on the condition of the fruit, the temperature, and the concentration of the chemical.

The experiments were repeated a number of times, and so far as possible with fruit in different degrees of susceptibility. The results have been fairly consistent, with no extremes of variation, except in

degree of injury due to period of exposure, concentration of chemical, or condition of the fruit used. Green fruit has shown the greatest sensitiveness to injury from the chemicals tested, and there is apparently a progressive decrease in this sensitiveness as the season advances and the fruit is more mature when shipped.

Injury to oranges, closely resembling the brown-stain disease, was produced by exposing fruit to the vapors of citral, citronellal, and various acetates, including ethyl, amyl, methyl, and octyl. (Pl. 3, F.) With a concentration of 1 to 2 ml of citral in an 8 to 10 liter desiccator, typical surface injury resembling the brown-stain disease was produced in a few hours. Longer exposures at 40° F. produced a similar effect, and the injury produced with low concentrations of citral and long exposure was indistinguishable from the stain on oranges resulting from cold storage. The acetates also were effective, but oranges were more resistant to their vapors than to those of citral.

Geraniol vapor caused no injury either to oranges or to grapefruit at low and moderate concentrations. Oranges were exposed for several days without apparent injury to the vapor of this alcohol at the concentration at which citral caused marked injury within a few hours.

Methyl anthranilate caused injury to oranges when the fruit was exposed to the concentrated vapor of the chemical for longer periods of time. With the concentration of citral doubled, three to five days were required to cause visible injury in the form of sunken and scalded areas in the peel. Linalool and *d*-limonene caused no injury to oranges or grapefruit at similar concentrations.

In both oranges and grapefruit, sunken lesions in the peel followed exposure to the vapor of acetaldehyde. These lesions were more nearly like those that characterize injury from low temperature than those produced by other chemicals. (Pl. 6, D.) Usually the lesions of typical storage spot originate in a collapse of the cells surrounding the oil vesicles. This is not invariably the case, however, for sometimes the oil glands show the initial effects of the disease. Some lesions produced with acetaldehyde began with the collapse of the cells above the oil vesicles, which was followed later by the collapse of the cells between the vesicles. Where only slight injury followed exposure of the fruit to the dilute vapor of acetaldehyde the effect was noticed only in the tissue directly above the glands.

When low concentrations of acetaldehyde were used the lesions formed on navel oranges resembled very closely those of the brown-spot disease. (Pl. 3, E.) When greater concentrations were used the entire surface of the fruit became scalded and pitted. With slightly green and very susceptible fruit the type of injury could be made to simulate brown spot or storage spot by varying the concentration and the period of exposure. Yellow ripe fruit that had been picked for several weeks was comparatively resistant to injury by this chemical. Fruits with smooth, fine-textured skin were most susceptible, but typical lesions were also produced on coarse thick-skinned fruits.

Lesions resembling those produced with acetaldehyde have developed also on fruit exposed to the vapor of acetic acid. One to two ml of 50 per cent acetic acid in an 8-liter desiccator affected oranges and grapefruit in very much the same way as did acetaldehyde. A mixture of acetaldehyde and acetic acid was more effective than either alone. In some of the earlier experiments, acetaldehyde that had

been stored for some time at room temperature was used, and fruit exposed to this lot was much more quickly injured than other lots exposed to acetaldehyde that had recently been purchased. Upon investigation it was found that the acetaldehyde had undergone oxidation and acetic acid had been formed in considerable quantity.

The color reactions of the tissues injured with acetaldehyde are very nearly the same as those that occur in the development of brown-spot and storage-spot lesions. The affected tissue is at first colorless, but, from a pale vinaceous shade, progressive color changes take place which proceed finally to the tones that characterize the natural lesions of these diseases. The purplish tinge so characteristic of the border portion of the brown-spot lesion on naval orange is especially noticeable around the margin of the injured tissue that develops after exposure to the vapor of acetaldehyde. On grapefruit the darker border of the lesion produced by acetaldehyde is very similar to that of the natural lesion.

DISCUSSION

In a preceding paper the writer (*13*) described certain functional diseases affecting vegetables in storage and transportation and showed by experiments that these diseases could be produced by exposing susceptible vegetables like potato, cabbage, and lettuce to conditions in which the quantity or the availability of oxygen was reduced. Other investigators had previously shown the liability of plants to injury at temperatures above the freezing point and had concluded that the injury was due to the accumulation of toxic substances as a result of incomplete cellular oxidations. Though the ultimate cause of the breakdown of the vegetables at low temperatures and under conditions of reduced oxygen tension was not determined, it was suggested that suboxidation (referring to respiration reactions) was of primary importance as a precursor of the toxicity actually causing the death of the cell. The occurrence of the blemish diseases of citrus fruits under similar conditions in storage and transportation, and the comparable reaction of these fruits to the conditions imposed in the vegetable experiments, show the qualitative relation of these blemishes and suggest again the rôle of suboxidation in their production. The circumstances that bring about reduced availability of oxygen in susceptible cells may be storage conditions of poor ventilation or low temperatures which prevent the utilization of oxygen requisite for normal respiration. The diseases of citrus fruits described as brown stain, brown spot, and storage spot thus appear to be manifestations of suboxidation reactions in the most active cells of the peel. It should be emphasized that the ultimate cause of each of these diseases is not yet known, although the results of some of the tests with chemicals may afford suggestive evidence.

Brown stain of orange is very similar in appearance to apple scald, and the experimental production of this disease with substances which produce scaldlike effects upon apple reveals the close similarity of the two disorders. The most typical injury was produced with the vapor of citral. The brown staining of the surface of orange which followed a short exposure to the vaporized substance was indistinguishable from the disease as it occurs naturally on oranges from cold storage.

The results of the experiment on air movement and the influence of temperature and of oiled wrappers seem to indicate that storage

spot and brown spot are probably not due to the surface action of poisons that can be dissipated by air currents or absorbed by neutral oils. The results of the oiled-wrapper experiments are, however, not conclusive enough to warrant an unqualified statement.

The manner in which the lesions of storage spot develop indicates that the initial site of injury is the deeper-situated parenchyma cells that circumscribe the oil vesicles. In the great majority of cases the cells over the oil glands do not, in the beginning, appear to be injured, and the breaking down of the peel tissues seems to originate in the subsurface parenchyma cells. Later, through desiccation of the cells surrounding the oil vesicles, the glands and the overlying cells are also affected. Such a sequence of events suggests that the initial injury occurs in deeper-situated cells and progresses outward.

The progressive increase in the permeability of the cells of the endocarp of orange at low temperatures indicates that such temperatures are injurious to the cell membranes. The most plausible explanation for this increase in permeability is the accumulation of poisonous substances within the cell. Since Pantanelli (*14*) demonstrated that at low temperatures an autodigestion of the proteins of orange occurs, it is evident that there is more than a theoretical basis for this explanation. Zoller (*24*) has shown that naringin, the bitter principle in the peel of grapefruit, gradually disappears during storage, and he suggests that certain flavors and "pink spots" which develop simultaneously with its disappearance are to be traced to the reactions of this glucoside. The products of the hydrolysis of naringin are glucose, rhamnose, and the phloroglucinol ester of *p*-hydroxy-cinnamic acid, and conceivably a gradual accumulation of this ester at low temperatures might be concerned in the occurrence of storage spot.

The causal factors in such functional diseases of apple as scald, internal browning, deep scald, etc., is a disputed question. These diseases develop in cold storage, and the most generally accepted view has been that of Brooks and his associates, who believe that injury to apples in the form of superficial scald is brought about by the action of poisonous substances like acetaldehyde and fruit esters which accumulate at low temperatures. The beneficial effects of ventilation and of oiled wrappers in preventing this type of breakdown lend support to this view. Thomas (*21*) and Trout (*23*), however, believe that the cells first become unhealthy from some cause as yet unknown and that following the initial injury zymasis occurs and the accumulated by-products accentuate the injury. According to this view acetaldehyde does not accumulate in apples or pears until the cells are injured from some other cause.

It is not proposed to consider here the controversial aspects of this problem. In the experiments with oranges and grapefruit, injuries resembling the lesions of brown spot and storage spot were produced by exposing these fruits to the dilute vapors of acetaldehyde and mixtures of acetaldehyde and acetic acid. Acetaldehyde is believed to be an intermediate product of respiration and under anaerobic conditions its production is increased (Kostyschew, Hübbenet, and Scheloumoff (*11*) and Thomas (*21*)). Of interest is the fact that acetaldehyde may be formed from citral in the presence of weak alkalies. Whether or not such a reaction might occur in citrus fruits in storage is not known. Since no analyses for acetaldehyde

in diseased tissues were made it can not be stated whether it is concerned in the causation of brown spot or storage spot. The fact that lesions resembling those of brown spot and storage spot can be produced by exposing fruit to dilute vapors of acetaldehyde or certain fruit esters suggests that some substance having a similar action may be concerned in the occurrence of these diseases at low temperatures or under conditions of poor ventilation.

Two hypotheses may be proposed for the injury to citrus fruits in cold storage. Low temperatures may cause a preponderance of hydrolytic activity resulting in excessive splitting of substances like glucosides, with a resultant accumulation of materials which poison the protoplasm. This explanation is supported by the partial control of pitting by prestorage treatment of grapefruit with kerosene-stove gas. This treatment speeds up katabolic processes greatly and must cause a marked decrease in storage materials like glucosides. The effects of accumulated products of incomplete respiration offers an alternative explanation for breakdown at low temperatures. Under normal conditions poisoning of the protoplasm is prevented by oxidation of toxic materials as fast as they are produced, but at low temperatures the oxidizing system in the cell is affected to such an extent that there may be a slow accumulation of these materials and eventual injury to the protoplasm. Low temperatures are evidently much more injurious to citrus fruits than to apples, since storage for 2 to 3 weeks at 32°–34° F. results in injury to grapefruit, whereas under similar conditions apples are not affected before 8 to 12 weeks.

The practical importance of oiled wrappers in the control of functional diseases of fruits in cold storage makes it desirable that conclusive evidence be obtained as to their value with oranges and grapefruit. The tests reported in this paper both with chemicals and wrappers should be repeated with fruit in condition likely to yield decisive results. In these experiments only market fruit was available and nothing was known of the previous treatment to which it had been subjected. More decisive results would probably follow a repetition of the experiments both with chemicals and oiled wrappers by using fruit freshly picked at a period when it was most susceptible to these blemish diseases.

SUMMARY

This paper reports the results of studies on certain functional diseases affecting citrus fruits in storage and transportation. The investigation deals chiefly with the storage spot or pox disease, but some experiments on brown spot and brown stain of orange are included.

The symptoms of these diseases are the result of a breakdown of the peel tissues following storage of fruit under conditions of poor ventilation or where the temperature is so low that normal respiratory functions are deranged.

Storage spot and brown spot have been produced in the laboratory under various conditions of air composition, and typical symptoms have followed storage of susceptible fruit in nitrogen for 4 to 10 days.

Under the conditions of these experiments air movement without renewal has not been effective in preventing storage spot at room temperature.

Storage spot has been produced at low temperatures where there has been no deficiency of oxygen in the surrounding air. Storage of green-skinned fruit for 10 to 14 days at temperatures close to 32° F. is sometimes sufficient to cause typical breakdown. Temperatures from 32°–42° are effective in producing breakdown of oranges and grapefruit where there is no oxygen deficiency in the surrounding air.

The susceptibility of oranges and grapefruit varies with the degree of maturity. Green fruit is usually more susceptible than colored and fully matured fruit.

Oiled wrappers have given variable results in the control of storage spot and brown spot. In most cases they have not decreased the amount of disease on either grapefruit or oranges in cold storage, but in a few instances they apparently have been beneficial.

Injury closely resembling brown stain has been produced on oranges by exposing them to the dilute vapors of certain chemicals. Citral has been especially effective in causing this type of injury. Similar injury has been produced with various acetates which also produce scald-like effects on apple.

Brown stain is a disease apparently very similar to apple scald with the injury incident in the surface cells and caused by the action of certain cumulative products of the orange itself.

Oranges and grapefruit exposed to the vapors of acetaldehyde, acetic acid, or a mixture of acetic acid and acetaldehyde have developed lesions in the peel resembling those characteristic of brown spot and storage spot. Other factors being equal, the degree of injury produced depends on the maturity of the fruit.

The lesions of brown spot and storage spot appear to arise through injuries in the parenchyma cells surrounding the oil vesicles. The effect of acetaldehyde and fruit esters upon oranges and grapefruit suggest that some substance having a similar action may be concerned in the causation or accentuation of the injury that follows storage of these fruits at low temperatures.

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EVAPORATION FROM SALT SOLUTIONS AND FROM OIL-COVERED WATER SURFACES[1]

By CARL ROHWER

Associate Irrigation Engineer, Bureau of Agricultural Engineering, United States Department of Agriculture

INTRODUCTION

Evaporation experiments performed at Fort Collins, Colo. (*6*)[2], and at various points in the western part of the United States at different altitudes, under a wide range of meteorological conditions, show that there is a definite relation between the evaporation and the difference in vapor pressure, the wind velocity, and the altitude.[3] There are, however, other factors, such as oil films on the water surface and the presence of various salts in the water, which are known to influence the evaporation from free-water surfaces.

The idea has been advanced that evaporation losses from reservoirs can be reduced materially by maintaining an oil film over the surface, and withdrawing the water for irrigation from below the reservoir surface without disturbing the oil film. Frequently irrigation water contains considerable quantities of salts in solution, and in experimental work it is sometimes desirable to add small amounts of salt to evaporation tanks in order to prevent the growth of algae in the water. Therefore it is important to know how the rate of evaporation from salt solutions and oil-covered water surfaces compares with that from fresh water.

In order to determine the effect of these factors on evaporation, and the influence of the meteorological factors under these conditions, experiments were performed at Fort Collins on the evaporation from oil-covered water surfaces, and from various solutions of sodium chloride and sodium sulphate. The results of these tests are reported in this paper.

EFFECT OF OIL ON EVAPORATION

In carrying on the observations to determine the effect of oil films on the evaporation from free-water surfaces, two Colorado land pans were installed under identical conditions at the hydraulic laboratory at Fort Collins, Colo. Both were filled with water, and the surface

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[2] Reference is made by number (italic) to Literature Cited p. 729.

[3] This relation is expressed by the formula:

$E=(1.465-0.0186B)(0.44+0.118W)(e_s-e_d)$, in which

E=evaporation in inches per 24 hours,

B=mean barometric pressure in inches of mercury at 32° F.,

W=mean ground wind in miles per hour,

e_s=mean vapor pressure of saturated vapor at temperature of water surface, in inches of mercury, and

e_d=mean vapor pressure of saturated air at the temperature of dewpoint, in inches of mercury.

The formula gives the evaporation from U. S. Weather Bureau, class A, land pans and floating pans, Colorado land pans, U. S. Bureau of Plant Industry land pans, U. S. Geological Survey floating pans, and other similar pans when the required data are substituted in it. For Fort Collins, Colo., elevation 5,000 feet, the factor $(1.465-0.0186B)$ is 1.000.

of one was covered with a film of oil. City water, which contained approximately 50 parts per million of solids, was used to fill the tanks. A comparison of the evaporation from the two tanks showed the effect of the oil film. The evaporation tanks were approximately 36 inches square and 18 inches deep, and were sunk in the ground within 1 inch of their tops. Both tanks were painted black with asphaltic paint. The water in the tanks was maintained at approximately the ground level, and a complete record of the meteorological conditions and of the evaporation was taken for each tank. The records consisted of the air and water temperature, the psychrometer reading and the evaporation loss for each tank, and the wind velocity and rainfall records which were the same for both tanks. The

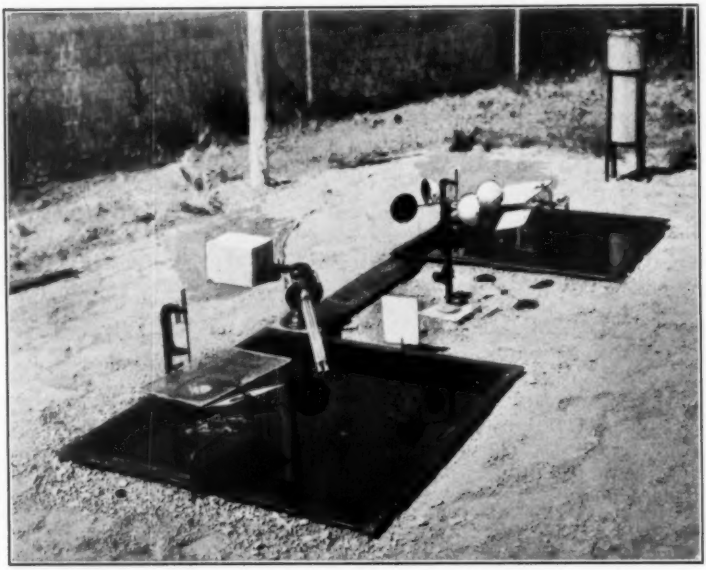

FIGURE 1.—Apparatus used in making tests on the effect of oil films on evaporation

evaporation tanks and the equipment used in making the observations are shown in Figure 1.

The water temperatures were taken at approximately one-half inch beneath the water surface, and the air temperatures were taken at about 1 inch above the water surface in each tank. The thermometers used in making the readings were of good quality and were graduated in degrees and in half degrees. They were compared with a thermometer graduated in tenths of a degree which had been calibrated by the Bureau of Standards, and were found to be quite accurate.

The wet and dry bulb readings for determining the vapor pressure of the water were made with a portable motor-driven aspiration psychrometer, as shown in Figure 1. The instrument was set so the air was drawn from a point about 1 inch above the water surface. This instrument had previously been compared with a calibrated psychrometer and was known to give accurate readings.

The evaporation loss was measured with micrometer hook gages reading to one-thousandth of an inch. These gages had a range of 1 inch, and consequently the variation in the level of the water was limited to this amount. Stilling wells were used in connection with the hook gages.

A standard Weather Bureau anemometer of the 4-cup Robinson type was used for determining the wind velocity. It was placed so that the cups were 18 inches above the ground, and in order to determine the ground velocity a conversion diagram, obtained by comparing the velocity at the 18-inch level with that at the ground level, was used.

The precipitation was measured in a standard Weather Bureau type rain gage which was located near the tanks, as shown in Figure 1.

The observations on the tanks were started on July 18, 1929, and were continued until October 28, 1929. The readings were taken twice daily: Between 7 and 8 a. m. and between 5 and 6 p. m. From these readings, the means of 24-hour periods, which began on the morning of one day and ended on the morning of the next, were computed. A summary of the results is given in Table 1.

Before the tests on the effect of oil on evaporation were started, observations were made under identical conditions on the evaporation from the free-water surfaces of the tanks to determine whether the rate of evaporation was the same from both tanks. These tests covered a period of 10 days, and showed (Table 1) that under identical conditions the rates of evaporation agreed quite closely, the mean difference for the period being only 2 per cent.

TABLE 1.—*Observed and computed evaporation from oil-covered water and from water in Colorado sunken tanks, the ratios between the evaporation from oil-covered water and from water, and the pertinent meteorological data at Fort Collins, Colo., 1929*

[Evaporation computed by formula $E=(1.465-0.0186B)\ (0.44+0.118W)\ (e_s-e_d)$]

Type of surface and tank used	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of oil-film evaporation to water evaporation observed [a]
		Air	Water				Observed	Computed [a]	
	1929	° F.	° F.	*Inch of mercury*	*Miles*	*Inch*	*Inch*	*Inch*	
Water, east tank	July 18 to	75.9	76.6	0.390	0.65	0.104	0.203	0.202	
Water, west tank	July 26	76.6	76.1	.376	.65	.104	.199	.194	
Water, east tank, 0.0002-inch oil film [b]	July 27 to	76.4	76.4	.467	1.08	0	.294	.266	
	Aug. 1	76.5	77.1	.481	1.08	0	.266	.275	0.919
Water, east tank, 0.002-inch oil film [b]	Aug. 13 to	77.7	75.5	.450	.63	.025	.227	.232	
	Aug. 21	78.7	83.7	.752	.63	.025	.074	.386	.333
Water, east tank, 0.002-inch oil film [b]	Aug. 22 to	73.7	73.7	.345	.91	.240	.194	.188	
	Aug. 29	75.2	79.4	.532	.91	.240	.080	.290	.440
Water, east tank, 0.004-inch oil film [b]	Sept. 10 to	57.5	60.2	.214	.90	.258	.116	.118	
	Oct. 5	59.3	64.8	.322	.90	.258	.026	.177	.279
Water, east tank, 0.002-inch oil film [c]	Oct. 6 to	46.8	50.9	.174	1.00	.245	.106	.097	
	Oct. 28	47.6	52.9	.208	1.00	.245	.065	.116	.597

[a] The computed evaporation and the ratio of the evaporation from the oil-covered water surface to that from the water surface are the means of the daily values.

[b] Transformer oil.

[c] Engine oil, medium.

After these tests had been completed, a film of transformer oil was added to one of the tanks. The first application consisted of 4 cc, which was equivalent to 0.0002 inch on the water surface. When first applied, the oil collected in pools on the water surface, and it took several days for it to spread out evenly. The effect of the film was so slight, however, that the surface was skimmed to remove the oil and then 40 cc of transformer oil was added, which was equivalent to 0.002 inch. This oil was immediately dissipated by the heavy rain occurring at the time, and it was not until tne rainfall ceased, when another application of the same amount of oil was made, that it was possible to determine the effect of the oil film. The 40 cc of oil caused a definite reduction in the evaporation, as shown in Table 1, but the effectiveness decreased as a result of the gradual disappearance of the oil film, and by the end of a week the oil was only slightly effective in reducing the evaporation. As soon as the evaporation from the oil surface was nearly equal to that from the free-water surface, the remaining oil was skimmed off and new oil added.

Several applications of oil were made at this rate, but for only two of these were the results free from the effect of heavy rains, which caused the oil to splash out of the tank and, in some cases, to overflow. For the two series of observations which were not affected by rains, the ratios of the evaporation from the oil-covered water surface to that from the free-water surface were 0.333 and 0.440, as shown in Table 1. An application of 80 cc of transformer oil, or a depth of approximately 0.004 inch, was more effective in reducing the evaporation and remained on the water surface longer. In this case, the ratio of the evaporation from the oil-covered surface to that from the water surface was 0.279 and this application of oil lost its effectiveness after 26 days, whereas the smaller quantity of oil was no longer effective after 10 days.

In order to test the effect of films of oil heavier than transformer oil, 40 cc of engine oil, medium grade, was applied to one of the tanks. The engine oil did not reduce the evaporation as much as the transformer oil, but it was not dissipated as rapidly and consequently was effective in reducing the evaporation for a longer period. As shown in Table 1, the ratio of the evaporation from the oil-covered water to that from the free-water surface was 0.597. The difference in the effect of the two different types of oil is probably due partly to the fact that the engine oil was applied late in the season when the evaporation from the free-water surface was small.

From the foregoing tests it is evident that it is possible to reduce the evaporation materially for short periods by the application of oil to the water surface. However, the rapidity with which the oil film disappears in a rainstorm, and the fact that the film is soon dissipated, even in dry weather, seems to make doubtful the economic feasibility of using oil for reducing evaporation from large water surfaces.

The formula

$$E = (1.465 - 0.0186B)\ (0.44 + 0.118W)\ (e_s - e_d)$$

shows the relation between the evaporation from a free-water surface and the various meteorological factors. In order to determine whether the same relation held for these experiments, the observed

meteorological data were substituted in the formula. The results are given in Table 1.

A study of the daily mean observed and computed evaporation from the free-water surface for each series of observations shows that the results agree quite closely, as a rule. The mean values for the entire period (not shown) are almost identical, the difference being only 2.9 per cent. The computed evaporation from the oil-covered tank, however, is greatly in excess of the observed evaporation and is considerably greater also than the observed evaporation from the free-water surface. That the computed evaporation from the oil-covered tank should exceed the observed evaporation is to be expected because of the retarding effect of the oil on evaporation, but the reason that it exceeds the evaporation from the free-water surface is that the temperature of the oil-covered water is materially higher than that in the free-water tank. This probably is due to the insulating effect of the oil, and to the reduction in the cooling of the water resulting from the smaller evaporation. The oil may also absorb more heat from the sun. The higher temperature increases the difference in vapor pressure, and since the computed evaporation is proportional to the difference in vapor pressure, it will obviously be greater than the observed evaporation.

EFFECT OF SODIUM CHLORIDE ON EVAPORATION

The effect of sodium chloride on evaporation was studied by comparing the evaporation from sodium chloride solutions of different concentrations with the evaporation from water under similar conditions. Three tanks identical in every respect, approximately 36 inches square and 18 inches deep, were used in carrying on the experiments. Each tank was painted black with asphaltic paint and was sunk in the ground to within 1 inch of the top, and the water level was maintained at approximately the ground level. The maximum variation in the water level was 1 inch, and as soon as the water reached the lower level the tanks were refilled. The evaporation tanks and the auxiliary equipment are shown in Figure 2.

The same observations were taken, the same instruments used, and the same procedure followed as in the experiments on the effect of oil on evaporation. One change, however, was made in the procedure when observations were taken on the more concentrated solutions because it was found that the density of the solutions in the stilling wells for the hook gages differed from that in the tanks, and, consequently, the water stood at a different level in the stilling wells than in the tanks. An attempt was made to correct this condition by making the inlets to the stilling wells larger, but this did not prove to be effective in eliminating the difficulty, so the stilling wells were removed entirely. Although the gages could not be read as accurately without the stilling wells, it was thought that the readings would be closer to the true conditions than they would if the stilling wells were used. A summary of the results of the observations is shown in Table 2.

Before the sodium chloride was added to the water, a series of observations covering 10 days was made to see whether the evaporation was the same from all the tanks when filled with water. As Table 2 shows, the evaporation from the different tanks for the 10-day period was nearly the same. The daily mean rates were not so

nearly similar, but the differences tended to balance each other, as is shown by the means for the period.

After completing the tests to determine whether the evaporation from all the tanks was the same when filled with water, common salt (NaCl) was added to the water in two of the tanks. The amounts of sodium chloride added were, respectively, 2 per cent and 5 per cent of the weight of the water in the tanks. Because the total weight of the solution was slightly greater than the weight of the water alone, the true percentage of salt in a given weight of solution was slightly less than that indicated.

The first series of tests on the comparison between the evaporation from water and from 2 and 5 per cent sodium chloride solutions was started June 19, 1930, and continued until July 22, 1930. At the end of this period, the 2 per cent sodium chloride solution was trans-

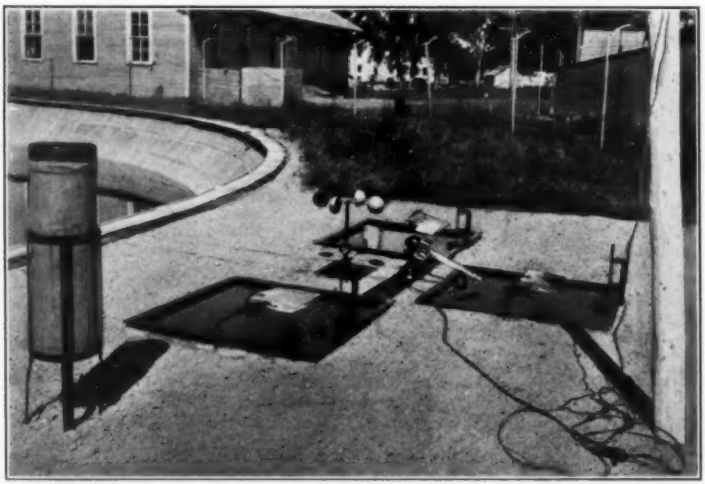

FIGURE 2.—Evaporation tanks and auxiliary equipment for testing the effect of sodium chloride on the evaporation from water

ferred to the tank which had been filled with water, and the tank which had held the 2 per cent solution previously was filled with fresh water. The experiments under this condition were continued until September 2, 1930, except for the period from August 11 to 18, 1930, during which the observations were discontinued on account of the heavy rainfall.

The results of the comparison for the first period show (Table 2) that the mean daily evaporation from the solutions increased as the salt concentration increased, or just the opposite of what was anticipated. The results of the comparison for the second period, when the fresh water and the 2 per cent sodium chloride solution were interchanged show that the evaporation from the salt solutions was less than that from the pure water; nevertheless the evaporation from the 5 per cent solution was greater than the evaporation from the 2 per cent solution.

For the entire period of 67 days, the mean daily evaporation from the different solutions, disregarding the position of the tanks, was,

respectively, 0.224, 0.219, and 0.223 inch for the pure water, the 2 per cent and the 5 per cent solutions. These results show that the evaporation was slightly less from the sodium chloride solutions, but still the loss from the 5 per cent solution was greater than that from the 2 per cent solutions, which indicates that the small differences noted are not significant.

While these observations were being carried on, it was observed that the temperatures of the solutions in the tanks containing sodium chloride seemed to be higher than the temperature of the water in the water tank. In the first series of tests the temperatures of the salt solutions were definitely higher than those of the water (Table 2), but in the second series the differences were insignificant. It is doubtful therefore whether the erratic results may be attributed entirely to the small differences in temperature.

TABLE 2.—*Observed and computed evaporation from water and from sodium chloride solution in Colorado sunken tanks, the ratios between the evaporation from sodium chloride solutions and from water, and the pertinent meteorological data at Fort Collins, Colo., 1930*

[Evaporation computed by formula $E=(1.465-0.0186B)(0.44+0.118W)(e_s-e_d)$]

Type of solution and tank used	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of solution evaporation to water evaporation observed [a]
		Air	Water				Observed	Computed [a]	
		° F.	° F.	*Inch of mercury*	*Miles*	*Inches*	*Inch*	*Inch*	
Water, west tank	June 8 to June 17.	70.8	70.6	0.425	1.18	0.645	0.259	0.248	.....
Water, south tank		70.4	70.6	.421	1.18	.645	.256	.246	.....
Water, east tank		69.8	71.0	.430	1.18	.645	.262	.252	.....
Water, south tank	June 19 to July 22.	75.8	74.3	.439	1.11	.956	.250	.251	.....
2 per cent NaCl, west tank		76.0	74.4	.445	1.11	.956	.254	.255	1.022
5 per cent NaCl, east tank		75.0	75.3	.442	1.11	.956	.255	.254	1.009
Water, west tank	July 24 to Sept. 2.	74.4	73.6	.373	.84	1.966	.197	.202	.....
2 per cent NaCl, south tank		74.1	73.3	.350	.84	1.966	.182	.189	.925
5 per cent NaCl, east tank		72.8	73.7	.350	.84	1.966	.190	.190	.955
Water	June 19 to Sept. 2.	75.1	73.9	.406	.98	2.922	.224	.227	.....
2 per cent NaCl		75.1	73.8	.398	.98	2.922	.219	.223	.974
5 per cent NaCl		74.0	74.5	.397	.98	2.922	.223	.222	.983
Water, west tank	Sept. 4 to Nov. 15.	50.2	52.4	.190	1.11	.574	.115	.108	.....
10 per cent NaCl, east tank		50.0	52.3	.170	1.11	.574	.109	.096	.930
20 per cent NaCl, south tank		49.9	52.2	.138	1.11	.574	.092	.079	.776

[a] The computed evaporation and the ratio of the evaporation from the sodium chloride solutions to that from the water are the means of the daily values.

Because the differences between the evaporation from water and that from 2 and 5 per cent solutions of sodium chloride were not significant, the salt content in the tanks was increased to 10 and 20 per cent in order to magnify the effects of the solutions on evaporation sufficiently so that they would not be overshadowed by observational errors. This was done by adding enough sodium chloride to that already in the tanks to obtain the desired concentrations. The tests on the concentrated solutions were started September 4, 1930, and were continued until November 15, 1930, when it became necessary to discontinue them on account of the cold weather.

Some difficulty was experienced with the 20 per cent solution because of its tendency to increase in concentration near the bottom. This phenomenon was detected when it was noticed that foreign material heavier than water sank to a definite level in the tank. Occasional stirring, however, corrected this condition.

The results obtained with the 10 per cent and 20 per cent solutions (Table 2) show that the sodium chloride materially reduced the rate of evaporation, and the smallest evaporation was from the tank containing the most concentrated solution.

The ratios of the daily evaporation from each of the tanks containing sodium chloride solutions to that from the tank containing water were computed, and the mean ratios for each series of observations are given in Table 2. These ratios were approximately 0.97 for the 2 per cent solution, 0.98 for the 5 per cent solution, 0.93 for the 10 per cent solution, and 0.78 for the 20 per cent solution. Experiments by Harris and Robinson (*2*) on the effect of common salt on the evaporation from glass tumblers, under laboratory conditions without wind, showed rates of evaporation from 10 and 20 per cent solutions, respectively, to be 79 per cent and 68 per cent of that from distilled water. Experiments by Briggs (*1, p. 193–195*) and by Lee (*3, p. 332–333*) showed similar results.

The agreement between the results obtained by the different experimenters is not close, but all the observations show that evaporation from the more concentrated salt solutions is definitely less than from weak solutions or from pure water.

In order to determine whether the relation between the evaporation from the different solutions of sodium chloride to the meteorological factors was the same as it was for water, the meteorological data were substituted in the formula

$$E=(1.465-0.0186B)\ (0.44+0.118W)\ (e_s-e_d),$$

which shows the relation between the meteorological factors and the evaporation from water in tanks. Before substituting the observed data in the formula, it was necessary to find the reduction in vapor pressure of the solution due to the salt in the water. According to Raoult's law (*5, p. 57–59*) the ratio of the vapor pressure of a solution to that of water is independent of the temperature and pressure. This fact is confirmed by the vapor-pressure data of the International Critical Tables (*4, p. 370–371*). In so far as determining the numerical values of the ratios is concerned, this law holds only for very dilute solutions and can not be used directly in the computation of the vapor pressures of concentrated solutions, but since the vapor pressures at the boiling point of water have been experimentally determined (*7, p. 182*) for different concentrations of various salts, the ratios can be determined by dividing the vapor pressure of the salt solution at the boiling point by the vapor pressure of the water at the same temperature. The vapor pressure of the solution at any other temperature is this ratio times the vapor pressure of water at the same temperature.

The values of the ratios computed on this basis are as follows: 2 per cent solution 0.989, 5 per cent solution 0.972, 10 per cent solution 0.948, and 20 percent solution 0.880. In computing the ratios, the true percentage of salt in the 10 and 20 per cent solutions, as determined by

the weight of salt in a given weight of solution, was used, but in the 2 and 5 per cent solutions the nominal percentage was used because the density of these solutions was not materially different from that of water. It will be noted that the reduction in vapor pressure was small for the 2 and 5 per cent solutions, which is probably the reason why the small percentages of salt had but a negligible effect on the evaporation.

The evaporation computed by substituting these data in the evaporation formula agrees quite well with the observed evaporation except in the last series of tests in which the 10 and 20 per cent solutions were used. Table 2 shows that the observed evaporation was greater than the computed evaporation for both the 10 and the 20 per cent solutions and also for the water, but the difference was greatest for the 20 per cent solution. The percentages of deviation are shown in Table 3.

TABLE 3.—*Deviation of computed from observed evaporation in the observations with solutions of sodium chloride*

Type of solution and tank	Mean evaporation per 24 hours		Deviation
	Observed	Computed[a]	
Water series:	*Inch*	*Inch*	*Per cent*
West tank	0.259	0.248	−4.2
South tank	.256	.246	−3.9
East tank	.262	.252	−3.8
2 and 5 per cent NaCl series:			
Water	.224	.227	+1.3
2 per cent	.219	.223	+1.8
5 per cent	.223	.222	−0.4
10 and 20 per cent NaCl series:			
Water	.115	.108	−6.1
10 per cent	.109	.096	−11.9
20 per cent	.092	.079	−14.1

[a] Computed by formula $E=(1.465-0.0186B)(0.44+0.118W)(e_s-e_d)$.

Although the percentages of deviation in the computed results for the 10 and 20 per cent solutions are rather large, it should be noted that the percentage for the water also is considerable and of the same sign. When this fact is taken into consideration, the differences between the observed and the computed rates of evaporation from the concentrated salt solutions do not appear remarkable; consequently it seems that the effects of meteorological factors upon evaporation from salt solutions are the same as upon evaporation from water.

EFFECT OF SODIUM SULPHATE ON EVAPORATION

The observations on the evaporation from solutions of sodium sulphate, commonly known as white alkali, were made with the same equipment as was used in making the observations on the effect of sodium chloride on evaporation. The same observations were taken and the same procedure was followed, except that the water-temperature readings were taken by holding the thermometer in each tank at the proper depth and the air temperatures were taken from the dry-bulb readings of the psychrometer. In conducting these tests, however, four tanks were used instead of three, and none of the tanks was painted at the beginning of the series of tests. No stilling wells were used in connection with the hook gages on the tanks because

it was assumed that the stilling wells would introduce errors into the readings on account of the difference in density of the solution in the tanks and in the stilling wells, as was noted in the case of the tests on sodium chloride solutions. Commercial anhydrous sodium sulphate was used in making the solutions. This salt is only moderately soluble in water at ordinary temperatures, and for this reason 10 per cent was the maximum concentration of solution investigated, but observations were also made on 2 and 5 per cent solutions to find out how effective the less concentrated solutions were in reducing the evaporation. As in the previous tests, the comparisons were made with the evaporation from water.

The use of four tanks instead of three made it necessary to rearrange the tanks. The new arrangement is shown in Figure 3. On account

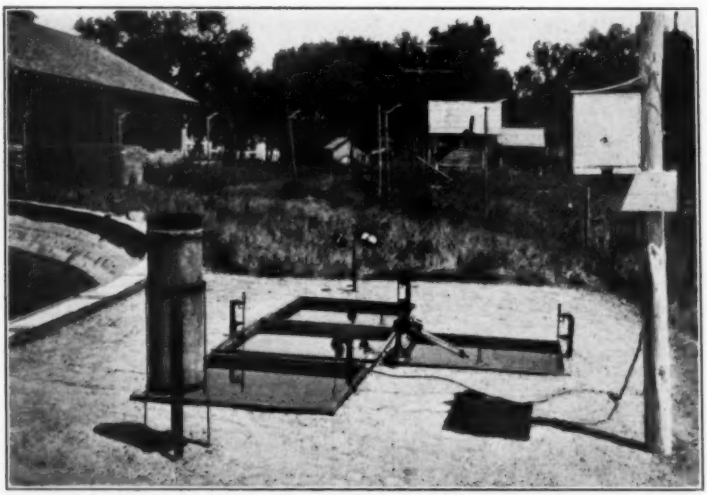

FIGURE 3.—Evaporation tanks arranged for making tests on the effect of sodium sulphate on evaporation from water

of the change in the position of the tanks, a preliminary series of observations was made when all the tanks were filled with water to determine whether the evaporation was the same from all of them. This series of observations was started on July 1, 1931, and was continued until August 1, 1931.

During the course of the observations it was noted that evaporation from the center tank of the three in a row was consistently less than that from the other tanks, and that the temperature of the water was also lower. This was attributed to the fact that the tanks had been left unpainted, because in the tests with sodium chloride solutions it was found that the action of the salt solution caused the paint to peel off, and as a result the interiors of the tanks were not of a uniform color. On July 16, 1931, the inside of each tank was painted with black asphaltic paint, and from then on the interiors of the tanks were uniform in color.

A summary of the results of these observations is given in Table 4. As shown in this table painting the tanks did not make the results

check any more closely. For the entire period that the tanks were filled with water only, the evaporation was, respectively, 0.296, 0.288, 0.304, and 0.294 inch. Comparison of these values with the mean for all the tanks shows that the evaporation from the center tank was 2.7 per cent low and that from the east tank was 2.7 per cent high, but the evaporation from the west and south tanks was almost identical with the mean value for all the tanks.

TABLE 4.—*Observed and computed evaporation from water and from sodium sulphate solutions in Colorado sunken tanks, the ratios between the evaporation from sodium sulphate solutions, and from water, and the pertinent meteorological data at Fort Collins, Colo., 1931*

[Evaporation computed by formula $E=(1.465-0.0186B)\ (0.44+0.0118W)\ (e_s-e_d)$]

Type of solution and tank	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of solution evaporation to water evaporation observed [a]
		Air	Water				Observed	Computed [a]	
		° F.	° F.	*Inch of mercury*	*Miles*	*Inches*	*Inch*	*Inch*	
Water, west tank	July 2 to 14	72.4	72.8	0.466	1.91	Trace	0.318	0.287	.....
Water, center tank		72.2	72.4	.462	1.91	Trace	.308	.284	.....
Water, east tank		72.4	73.1	.480	1.91	Trace	.326	.294	.....
Water, south tank		72.1	72.8	.474	1.91	Trace	.313	.291	.....
Water, west tank	July 16 to 31 [b]	78.1	76.6	.469	1.60	0.120	.279	.284	.....
Water, center tank		77.7	76.2	.463	1.60	.120	.272	.279	.....
Water, east tank		78.0	76.6	.476	1.60	.120	.287	.286	.....
Water, south tank		77.8	76.5	.475	1.60	.120	.278	.286	.....
Water, west tank	July 2 to 31	75.6	74.9	.468	1.74	.120	.296	.285	.....
Water, center tank		75.2	74.6	.462	1.74	.120	.288	.281	.....
Water, east tank		75.4	75.0	.478	1.74	.120	.304	.290	.....
Water, south tank		75.2	74.8	.474	1.74	.120	.294	.288	.....
Water, south tank		71.1	72.2	.412	1.12	.886	.220	.231	.....
2 per cent Na_2SO_4, west tank	Aug. 1 to 31	71.2	72.0	.385	1.12	.886	.227	.216	1.030
5 per cent Na_2SO_4, center tank		71.1	71.7	.380	1.12	.886	.209	.213	.955
10 per cent Na_2SO_4, east tank		71.2	72.1	.391	1.12	.886	.215	.219	.984
Water, south tank		64.4	65.4	.320	1.29	.369	.186	.188	.....
2 per cent Na_2SO_4, west tank	Sept. 1 to 30	64.4	65.2	.313	1.29	.369	.191	.182	1.020
5 per cent Na_2SO_4, center tank		64.3	65.1	.298	1.29	.369	.179	.174	.945
10 per cent Na_2SO_4, east tank		64.4	65.2	.306	1.29	.369	.185	.178	.972
Water, south tank		49.1	50.7	.162	1.78	1.013	.100	.098	.....
2 per cent Na_2SO_4, west tank	Oct. 1 to 31	49.3	50.5	.159	1.78	1.013	.108	.096	1.087
5 per cent Na_2SO_4, center tank		49.2	50.4	.152	1.78	1.013	.108	.092	1.028
10 per cent Na_2SO_4, east tank		49.1	50.4	.151	1.78	1.013	.106	.092	.974
Water, south tank		61.5	62.7	.298	1.40	2.268	.168	.172	.....
2 per cent Na_2SO_4, west tank	Aug. 1 to Oct. 31.	61.6	62.6	.285	1.40	2.268	.175	.164	1.045
5 per cent Na_2SO_4, center tank		61.5	62.4	.277	1.40	2.268	.165	.160	.976
10 per cent Na_2SO_4, east tank		61.5	62.5	.282	1.40	2.268	.168	.163	.977

[a] The computed evaporation and the ratio of the evaporation from the sodium sulphate solutions to that from the water are the means of the daily values.

[b] Tanks cleaned and painted with asphaltic paint on July 16.

The sodium sulphate was added to the tanks on August 1, 1931. The amount of salt used was determined by taking the indicated percentage (2, 5, or 10) of the weight of water in each tank as determined from its volume. This method gave only approximate results. The true percentages of sodium sulphate in the solutions were determined from their temperatures and densities. No sodium sulphate was added to the south tank because the comparative results on the evaporation from water were to be obtained from it.

The observations on the sodium sulphate solutions were started on August 1, 1931, and were continued until November 1, 1931. The results are given in Table 4, which is a summary by months of the

daily observations. The results in Table 4 show that the evaporation from the 10 per cent solution was greater than it was from the 5 per cent solution in August and September, and less in October, but in every case the differences were small. In this connection it should be recalled, however, that when these tanks were filled with water, the evaporation from all the tanks was not the same, the center tank being consistently low and the east tank consistently high. As the 5 per cent solution was in the center tank and the 10 per cent solution was in the east tank, one reason for the unexpected results in the August and September records becomes apparent.

No doubt the principal reason that the same results were not obtained in October was that conditions during this period were unusual. The temperatures in the tanks dropped so low that the 10 per cent solution reached the saturation point and started to crystallize. Some of the crystals formed as flakes on the surface of the solutions and probably reduced the evaporation to some extent by reducing the evaporating area. When the temperature dropped considerably below freezing, the crystallization of the solution apparently was carried below the saturation point, for it was observed that ice formed only on the tank containing the 10 per cent solution and on the tank containing water. Although all the tanks containing the sodium sulphate solutions were stirred each morning and evening when the readings were taken, the fact that ice formed only on the tank containing the 10 per cent solution and on the tank containing water indicated that under these conditions, at least, the surface of the solution was nearly free from sodium sulphate.

At the end of the season, observations made on the densities of the solutions showed that on account of the crystallization of the sodium sulphate, the specific gravity of the solution in the tank containing 10 per cent of sodium sulphate had dropped until it was only slightly higher than the specific gravity of the 5 per cent solution. Under normal conditions this should have increased the evaporation, but the accelerating effect was probably counterbalanced by the retarding effect of the ice in the tank. As the weather grew colder, there was also a tendency for the solutions to crystallize around the edges of the tanks. The capillary action kept these crystals wet, which increased the area of the evaporating surface and consequently increased the evaporation. This is probably the reason why the evaporation from the solutions was consistently greater than it was from the water in October. In this connection it should, however, be mentioned that the ice in the water tank during the latter part of October caused a decrease in the evaporation, which would also make the difference between the evaporation from the water and from the solutions greater.

For the entire period of three months, the mean daily evaporation from the water and from the 2, 5, and 10 per cent solutions was, respectively, 0.168, 0.175, 0.165, and 0.168 inch, and the ratios of the evaporation from the different solutions to that from water were, respectively, 1.045, 0.976, and 0.977. These results are inconsistent because the evaporation from the tank containing water is exactly the same as the evaporation from the tank containing 10 per cent of sodium sulphate and less than the evaporation from the tank containing 2 per cent of sodium sulphate. The differences are small, however, and indicate that the effect of sodium sulphate on the evaporation, for these conditions at least, is negligible (*1, p. 195*). Correct-

ing the results in accordance with the differences found when making the observations on the tanks when all were filled with water would make the results more consistent but would not show that the sodium sulphate affected the evaporation materially.

The relation of the meteorological factors to the evaporation from the sodium sulphate solutions was checked by substituting the observed meteorological data in the formula

$$E=(1.465-0.0186B)\ (0.44+0.118W)\ (e_s-e_d),$$

which shows the relation between the evaporation from free-water surfaces of this type and the meteorological data. If the same relation holds, then the evaporation computed by the formula should equal the observed evaporation. Before substituting the data in the formula, it was necessary to compute the vapor pressure of the solutions. This was done in the same manner as for the sodium chloride solutions and was based on the true percentages of sodium sulphate in the solutions, as determined from their densities. The results obtained by substituting these data in the formula are given in Table 4 and are summarized in Table 5.

TABLE 5.—*Deviation of computed from observed evaporation in the observations with solutions of sodium sulphate*

Type of solution, month and tank	Mean evaporation per 24 hours		Deviation
	Observed	Computed[a]	
Water series:			
July:	*Inch*	*Inch*	*Per cent*
South tank	0.294	0.288	−2.0
West tank	.296	.285	−3.7
Center tank	.288	.281	−2.4
East tank	.304	.290	−4.6
2, 5, and 10 per cent solutions:			
August:			
Water	.220	.231	+5.0
2 per cent	.227	.216	−4.8
5 per cent	.209	.213	+1.9
10 per cent	.215	.219	+1.9
September:			
Water	.186	.188	+1.1
2 per cent	.191	.182	−4.7
5 per cent	.179	.174	−2.8
10 per cent	.185	.178	−3.8
October:			
Water	.100	.098	−2.0
2 per cent	.108	.096	−11.1
5 per cent	.108	.092	−14.8
10 per cent	.106	.092	−13.2
August, September, and October means:			
Water	.168	.172	+2.4
2 per cent	.175	.164	−6.3
5 per cent	.165	.160	−3.0
10 per cent	.168	.163	−3.0

[a] Computed by formula $E=(1.465-0.0186B)\ (0.44+0.118W)\ (e_s-e_d)$.

These results show that it was possible to compute the evaporation from sodium sulphate solutions by substituting the wind velocity, barometric pressure, and difference in vapor pressure in the formula for the evaporation from water. The agreement between the observed and computed evaporation for the sodium sulphate solutions is not so close as it is for the water, but the errors are not large except for the October observations. For this series, the deviation may be due

to the fact that the solutions started to crystallize on the edges of the tanks, thus increasing the area of the evaporating surface as a result of the capillary action of the crystals. The means for the entire period during which the observations were made on the solutions show a close agreement, but the results are necessarily affected by the large errors occurring in the October observations. If the October observations are disregarded, the results show that the same law holds for both the sodium sulphate solutions and the water.

SUMMARY

Observations on the effect of oil films on evaporation show that evaporation is reduced by the presence of the oil film, and that the effectiveness of the film increases with its thickness. Heavy oil apparently did not reduce the evaporation as much as light oil, but this may have been due to the fact that the evaporation from the water surface was small at the time the observations on heavy oil were made. The short time in which the oil films were effective in reducing evaporation, due to dissipation by rainfall, wind, and other causes, indicates that this method is not economically feasible for reducing evaporation from large water surfaces. The relation which holds between the meteorological factors, that is, barometric pressure, wind velocity, and difference in vapor pressure, and the evaporation from free-water surfaces does not hold for water surfaces covered by oil films. The temperatures of oil-covered water surfaces are definitely higher than those of free-water surfaces similarly exposed.

Observations on the evaporation from solutions of sodium chloride of different strengths, when compared with the evaporation from water surfaces, all in exactly similar tanks similarly exposed, show that the evaporation from 2 and 5 per cent solutions does not differ materially from that from water, but that the evaporation from 10 and 20 per cent solutions is definitely less than that from water. For the 10 and 20 per cent solutions the evaporation decreases as the salt concentration increases. For the entire period of the observations, the ratios of the evaporation from the 2, 5, 10, and 20 per cent sodium chloride solutions to that from water were respectively 0.97, 0.98, 0.93, and 0.78.

The relation between the rate of evaporation from water and the meteorological factors evidently holds also in general for sodium chloride solutions. If the vapor pressure is corrected for the effect of the sodium chloride, the computed rates of evaporation agree well with the observed rates, but the agreement is closer for the weaker than for the more concentrated solutions.

The evaporation from the sodium sulphate solution agrees quite closely with the evaporation from water when exposed under similar conditions. For the entire period during which the observations were taken, the ratio of the evaporation from the 2 per cent solution to that from water was 1.04, and from the 5 per cent and 10 per cent solutions was 0.98. These ratios show no definite trend, but observations made on the same tanks when filled with water showed that the evaporation from the tank which was later filled with the 5 per cent sodium sulphate solution was low, and that the evaporation from the tank which was later filled with the 10 per cent solution was high.

In general, the evaporation from the sodium sulphate solutions follows the same law as the evaporation from water. In computations of evaporation, of course, the vapor pressure of the water in the solution should be corrected for the effect of the salt. As in the case of the sodium chloride solutions, the agreement between observed and computed evaporation was closer for the larger than for the smaller rates.

The evaporation law expressed by the formula

$$E=(1.465-0.0186B)\ (0.44+0.118W)\ (e_s-e_d)$$

was found to be true for the evaporation from the tanks containing water throughout the entire series of tests.

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THE EFFECT OF LATITUDE, LENGTH OF GROWING SEASON, AND PLACE OF ORIGIN OF SEED ON THE YIELD OF COTTON VARIETIES[1]

By G. A. HALE

Assistant Agronomist, Georgia Agricultural Experiment Station

INTRODUCTION

The determination of the inherent yielding ability of seed from different cotton varieties under local field conditions is one of the projects in agronomy underway at all of the Cotton Belt experiment stations. According to Cook (*2, p. 11*)[2] there are probably from 200 to 300 named varieties available for testing. These so-called varieties were developed in various ways, and under a wide range of growing conditions. The multiplicity of environmental factors affecting yield, such as soil, prevalence of insects and diseases, amount of light, sunshine, heat, moisture, length of growing season, place of testing, and the conditions under which the seed were produced, make an analysis of cotton varietal yields difficult even for a single location.

Very little work has been reported on the effect of latitude, length of growing season, and place of origin of seed on the yield of cotton varieties. Winters, as reported by Brown (*1, p. 236*), selected and increased a strain of the King variety of cotton at West Raleigh, N. C., for two years. In 1916 the selected seed was divided, and one half was grown at West Raleigh and the other half at Agricultural College, Miss. During 1917 and 1918, the Mississippi-grown seed was compared with the North Carolina-grown seed at both places. At Agricultural College, Miss., it was found that seed grown there produced taller plants and more bolls than did seed from the original lot grown at West Raleigh, N. C. In the corresponding tests made in North Carolina, Mississippi-grown seed also made the best growth and slightly better yields.

MATERIALS AND METHODS

The yield data for as many varieties and locations as were available for recent years were used in this study. Seasonal averages of not less than three years for lint or seed cotton yields were obtained from published reports or were compiled from them and from type sheets furnished by those in charge of cotton-variety testing in Texas, Oklahoma, Missouri, Arkansas, Louisiana, Mississippi, Tennessee, Alabama, Georgia, and South Carolina. The source given for each variety during the years making up the average yields may not be exact, as in some cases it was necessary to assume that seed was obtained from the same place every year.

The approximate latitude of the places of origin of the seed and of the places where the tests were made was determined from maps (*3*) giving the latitude to one-half degree, except for Texas where the

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[2] Reference is made by number (italic) to Literature Cited, p. 737.

latitude was given in a whole degree, or approximately 70 miles. The approximate average number of frost-free days, or the average number of days free of killing frost, at the places of origin and places where tested was determined from a map (*7, p. 11*) prepared by the United States Weather Bureau. The data are not presented for the 37 test locations as the correlations given summarize the results of this phase of the work.

The map (fig. 1) of the United States Cotton Belt shows the location of varietal experiments and the approximate number of annual frost-free days for each location included in the correlation study.

Two simple correlation coefficients were calculated to express mathematically the tendency of varieties with high yields to have places of origin of seed similar in latitude and length of growing season to those where the tests are made. Relationships were studied between the relative yield (highest-yielding variety equaling 100 per cent) of each variety and the difference in latitude, and the difference in the annual number of frost-free days, at each of the 37 test locations, and the place of origin of the seed, of each variety tested. The number of varieties at each location varied from 4 to 26, making it possible to include 406 sets of observations in each correlation study.

In order to get additional information on the adaptation of cotton seed, another study was made in which the yield of a local or unnamed variety in 68 tests was compared with the average yields of introduced seed of named varieties. The results of this study are given in Table 1.

TABLE 1.—*Comparison of yield from introduced and locally grown cottonseed in 68 tests at 37 locations*

Place tested	Year	Varieties tested	Rank of local seed in yield	Acre yield of—				Difference in favor of local seed	
				Local seed		Average all varieties			
				Lint	Seed cotton	Lint	Seed cotton	Lint	Seed cotton
		Number		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Burdette, Ark	1920	22	1		1,083		866		+217
Fort Smith, Ark	1920	8	1		1,210		1,070		+140
Do	1922	8	4		475		468		+7
Baber, Ark	1920	8	5		887		888		−1
Heber Springs, Ark	1920	8	4		1,427		1,403		+24
Do	1921	10	4		303		335		−32
Blytheville, Ark	1922	8	5		1,537		1,571		−34
Wynne, Ark	1922	8	5		633		706		−73
Waldron, Ark	1922	8	1		528		277		+251
Gregory, Ark	1922	8	1		1,110		998		+112
Do	1923	13	8		273		368		−95
Do	1924	17	15		1,103		1,361		−258
Morrilton, Ark	1920	7	4		1,993		1,917		+76
Brickeys, Ark	1920	8	5		860		893		−33
Dermott, Ark	1920	8	7		790		953		−163
Marianna, Ark	1921	9	3		1,553		1,182		+371
Bryant, Ark	1921	8	5		536		548		−12
Do	1921	7	2		672		516		+156
Magnolia, Ark	1921	8	9		285		359		−74
Bonnerdale, Ark	1922	8	6		334		358		−24
Pocahontas, Ark	1922	8	1		1,300		911		+389
Robroy, Ark	1923	19	13		779		872		−93
Laurel Hill, Fla	1928	5	4		867		989		−122
Do	1930	19	6	314		311		+3	
Sylacauga, Ala	1928	26	11	159		152		+7	
Do	1929	32	14	269		257		+12	
Cedar Bluff, Ala	1928	26	22	325		405		−80	
Do	1929	32	5	425		358		+67	
Belle Mina, Ala	1928	26	10	456		432		+24	
Do	1930	33	16	243		244		−1	
Do	1931	28	6	457		408		+49	

TABLE 1.—*Comparison of yield from introduced and locally grown cottonseed in 68 tests at 37 locations*—Continued

Place tested	Year	Varieties tested	Rank of local seed in yield	Acre yield of—				Difference in favor of local seed	
				Local seed		Average all varieties			
				Lint	Seed cotton	Lint	Seed cotton	Lint	Seed cotton
		Number		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Alexandria, Ala.	1928	26	5	173		145		+28	
Do.	1930	33	20	218		226		−8	
Do.	1931	28	12	328		323		+5	
Andalusia, Ala.	1928	19	16	283		320		−37	
Do.	1929	19	2	343		309		+34	
Do.	1930	22	8	174		162		+12	
Do.	1931	21	5	519		569		−50	
Crossville, Ala.	1929	32	7	446		417		+29	
Do.	1930	33	14	446		428		+18	
Do.	1931	28	18	606		628		−22	
Auburn, Ala.	1929	20	15	275		292		−17	
Do.	1930	27	8	381		364		+17	
Do.	1930	33	12	503		469		+34	
Do.	1931	34	3	527		452		+75	
Do.	1931	19	6	376		360		+16	
Prattville, Ala.	1929	19	7	382		366		+16	
Do.	1930	22	3	212		186		+26	
Do.	1930	33	4	212		161		+51	
Do.	1931	28	16	504		507		−3	
Do.	1931	19	8	496		491		+5	
Benton, Ala.	1929	19	1	131		98		+33	
Do.	1930	22	8	394		367		+27	
Do.	1931	20	3	426		507		−81	
Headland, Ala.	1929	19	6	385		369		+16	
Do.	1930	24	3	628		574		+54	
Do.	1931	21	2	274		335		−61	
La Fayette, Ala.	1930	33	13	379		348		+31	
Do.	1931	27	23	462		394		+68	
Benton, Ala.	1930	22	10	326		322		+4	
Aliceville, Ala.	1931	19	14	552		529		+23	
Do.	1931	28	20	536		569		−33	
Monroeville, Ala.	1931	19	15	366		321		+45	
Gastonburg, Ala.	1931	28	5	448		503		−55	
Rome, Ga.	1931	25	5	390		338		+52	
Fort Valley, Ga.	1931	23	9	573		555		+18	
Carnegie, Ga.	1931	23	11	379		384		−5	
Waynesboro, Ga.	1931	23	17	344		376		−32	
Average		20	8	379±13	893±65	370±13	861±60	9±18	32±88

EXPERIMENTAL RESULTS

CORRELATIONS BETWEEN YIELD AND LATITUDE AND YIELD AND NUMBER OF FROST-FREE DAYS

Correlation coefficients of −0.143 and −0.118 were found between relative yield and differences in latitude and differences in number of frost-free days, respectively. While the coefficients are small, the large number of observations justify the conclusion that there is a significant correlation between the yield of cotton varieties and their place of origin.

According to the tables prepared by Wallace and Snedecor (*8, p. 63*), when the number of observations is 400, the least significant value of r is 0.098, while 0.128 is the least highly significant value. The negative coefficients show that high relative yield is associated with small differences between the latitude and the annual number of frost-free days for the test locations and the places of origin of seed of the varieties tested.

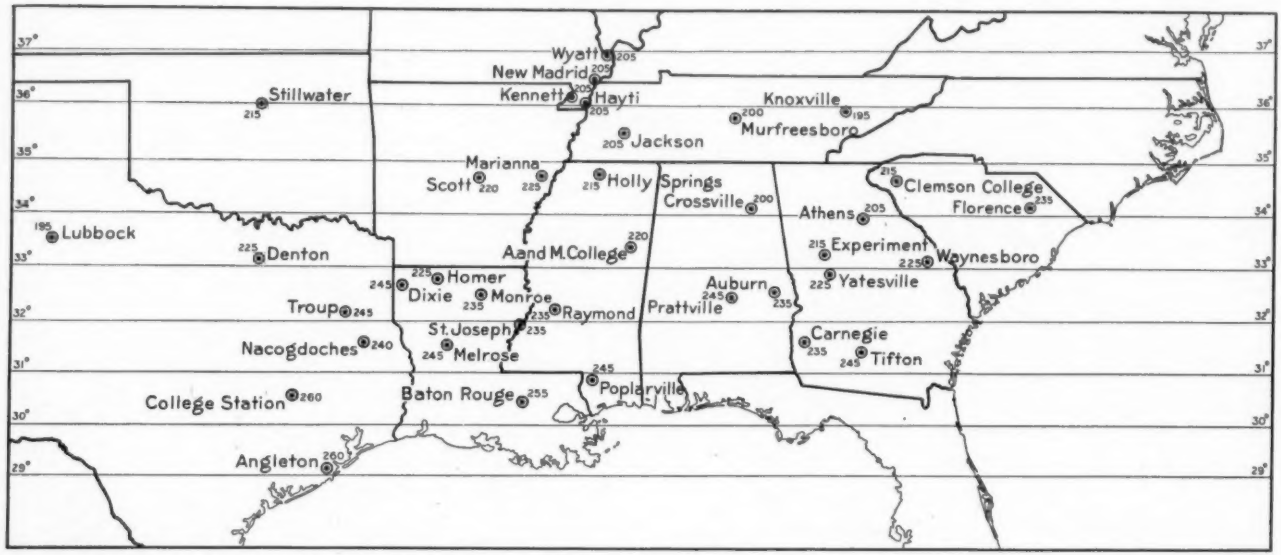

FIGURE 1.—United States Cotton Belt showing location, and approximate number of frost-free days for experiments used in the correlation study

COMPARISON OF LOCAL AND INTRODUCED COTTONSEED

Table 1 gives the average yield of all named or introduced varieties and the yield and rank of the locally grown cottonseed of unnamed kinds in 68 varietal tests at 37 places in Arkansas, Florida, Alabama, and Georgia. The differences in favor of the yield of local seed for each location is given as well as the average yields for all tests where local and introduced seed are compared. The average acre yields of seed cotton in 23 experiments are reported, and average yields of lint in 45 experiments. The yield of local or home-grown seed exceeded that of all varieties by 32 ± 88 pounds per acre of seed cotton and by 9 ± 18 pounds per acre of lint. In 41, or 60.3 per cent, of the 68 tests tabulated, the locally grown seed outyielded that brought in from other places and latitudes for the experiments. The average number of varieties in each test was 20, and the average rank of local seed in yield was 8.

DISCUSSION

The correlation coefficient of -0.143 between the relative yield and the difference in latitude of the place of origin and location of the varietal test may be considered highly significant. It shows that there is a strong tendency for cotton to produce high yields when the seed is grown in the same latitude as the place where it is tested. No data are available as to the length of time necessary for this climatic adaptation, but it is probable that at least three or four years are necessary.

The correlation coefficient of -0.118 between the relative yield and the difference in frost-free days at the place of origin and the location of the varietal test may be considered as significant. This coefficient indicates that the number of frost-free days where varieties originate has about as much effect on their yielding ability as the latitude at the place of origin. This would be expected, as the length of the growing season largely determines the amount of heat and light available for plant growth. This should have as much influence on varietal characters as the distance from the equator, because of differences in altitude and other conditions. For example, Figure 1 shows that Crossville, Ala., and Florence, S. C., are located at approximately the same latitude; yet the annual average number of frost-free days at Crossville is approximately 35 days less than at Florence.

In interpreting the data presented in Table 1 in which comparisons are made between the yield of local and introduced cottonseed, it should be remembered that the local seed of unnamed varieties were in most instances badly mixed and considered undesirable for planting purposes. The introduced seed were of so-called purebred varieties from the best cotton breeders. While the average difference in yield in favor of home-grown seed is not statistically significant, it certainly suggests that there is an advantage in growing cotton from seed produced under local conditions.

The importance of natural selection in cotton breeding, and the part that local climatic conditions play in the acclimatization and adaptation of a local or introduced cotton variety, are probably not fully appreciated by many who think the methods and skill of cotton breeders largely determine their success in the breeding of locally and widely adapted cotton varieties. A correlation study (*6, p. 10*) showed no significant relationship between the yield of cotton plant

selections and their progeny, although most of the present cotton varieties were developed by the plant-to-row method.

Angleton, Tex., approximate latitude 29° 15′, located in the extreme southern edge of the Cotton Belt, has an average length of day of about 14.1 hours on June 21 (*4, p. 10*) while Wyatt, Mo., with an approximate latitude of 36° 45′, located in the extreme northern edge, has about 14.8 hours of daylight on the same date. The difference in the length of day and the total amount of light available for plant use at the various latitudes included in this study is not great, but as the cotton plant is very sensitive to low temperatures, there is a difference of about two months in planting dates at the southern and northern edges of this territory.

The cotton plant is sensitive to sunlight and does not fruit well without an abundance of it. The percentage of the total possible amount of sunshine (*9*) during the growing season for the Cotton Belt varies more with changes in longitude than with latitude. Oklahoma and west Texas get from 70 to 80 per cent while much of Georgia and Alabama get only 50 to 55 per cent of the total or possible amount of sunshine during the summer. None of the Texas varieties tested east of that State made high yields. It is likely that differences in amount of sunshine in the eastern and western parts of the Cotton Belt account in part for the poor adaptation of western cottons to eastern conditions.

The amount of rainfall for the Cotton Belt (*5, p. 26–27*) also varies more with changes in longitude than with latitude, but not so much as does the amount of sunshine. The eastern part of the Cotton Belt and much of the territory along the Gulf coast has an average summer precipitation of from 14 to 19 inches, while most of the central and western parts get only about 8 to 14 inches of rainfall during the three summer months.

Another variable in growing conditions at different locations in the Cotton Belt is the infestation and damage by the cotton boll weevil (*Anthonomus grandis*). The length of growing season as measured by the number of frost-free days may be much greater in southern latitudes and at low altitudes than in northern latitudes and at high altitudes, but because of the nature of boll-weevil activities the cotton-fruiting period is often about the same length of time. Much of the northern part of the Cotton Belt is free, or practically so, of boll-weevil damage, while the southern half and most of the Atlantic Coastal Plain is infested with boll weevils every year. Boll-weevil infestation tends to shorten the normal length of fruiting period by preventing cotton from setting and maturing bolls during the latter part of its normal fruiting period. Varieties bred for short growing seasons in northern latitudes may sometimes be better adapted to southern latitudes than are varieties bred for long growing periods, especially during seasons favorable to the boll weevil.

The differences in climatic, boll-weevil, and soil conditions at different locations in the Cotton Belt may explain, at least in part, the observed tendency for locally grown seed to outyield seed from distant places.

SUMMARY

The approximate latitude and number of frost-free days was determined for 37 locations where cotton varietal tests have been conducted for a period of at least three years. The same data, the relative yield,

the average annual yield, and the place of origin of seed was determined for the varieties tested at each. A correlation coefficient of −0.143 for relative yield and differences in latitude at the test location and place of origin was found. A coefficient of −0.118 for relative yield and differences in number of frost-free days at test location and place of origin was found.

Information on the comparative yielding ability of local and introduced cottonseed in 68 experiments located in four States is given. Locally grown seed outyielded introduced seed in 60.3 per cent of the places where the average yield of all named varieties was compared with the yield of local seed.

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THE HAIRY-VETCH BRUCHID, BRUCHUS BRACHIALIS FAHRAEUS, IN THE UNITED STATES [1]

By J. C. BRIDWELL, formerly *Specialist in Bruchidae and Their Parasites, Division of Stored Product Insects, Bureau of Entomology,* and L. J. BOTTIMER, *Assistant Entomologist, Food and Drug Administration, United States Department of Agriculture*

INTRODUCTION

The genus Bruchus was restricted by Schilsky (*6*) [2] to include only the immediate allies of *Bruchus pisorum* (L.), the pea weevil, of which he tabulated 24 species known to him. To these may be added others doubtfully distinct, imperfectly known, or more recently described, which increase the nominal species of the genus to a total of about 46. All these species are native to the Palearctic region. Two of them are already well known in the United States as major pests of the plants affected. *B. pisorum* was the first of the genus and one of the first species of the family to be recognized. It was described in 1752, and was recorded as having destroyed in the 1740's the flourishing colonial American industry of producing dry peas for ships' stores. *B. rufimanus* Boheman, the broadbean weevil, has now practically destroyed the broadbean industry of California. A third species, *B. brachialis* Fahraeus, has in recent years gained a foothold in this country, for in 1931 the junior author found it heavily infesting the seeds of vetches growing in New Jersey, Delaware, Maryland, and North Carolina, and in 1932 it was found in Virginia. In view of these facts it seems wise to present a brief summary of the knowledge at present available of the habits of the members of this genus and to point out the increased danger of their establishment in the United States as a result of changed commercial conditions.

FOOD PLANTS OF BRUCHUS (RESTRICTED) [3]

All species of Bruchus of which we have credible records [4] feed as larvae in seeds of plants of the leguminous tribe Vicieae, infesting the pods in the field before they ripen. They are unable to reinfest the seed in storage.

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[1] Received for publication June 30, 1932; issued May, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 750.

[3] Unless otherwise indicated, the records of host plants of the species herein discussed are all based upon determinations, made by the senior author of insects associated with their host seeds contained in the collections of the U. S. National Museum. A large part of this material was obtained in routine inspection work by officers of the Federal Horticultural Board and its successor, the Plant Quarantine and Control Administration (now the Bureau of Plant Quarantine), of the U. S. Department of Agriculture. Much of the seed material was imported by the Division of Foreign Plant Introduction for the experimental work of the Bureau of Plant Industry. The determination of the seeds has been verified by Roland McKee, in charge of investigations of the vetchlike plants, or by H. C. Skeels, in charge of the seed collection of the Division of Foreign Plant Introduction. To both of these men the writers are indebted for assistance in botanical and agricultural matters in the preparation of the present work.

[4] Records of species affecting the seeds of Lotus, Ulex, Phaseolus, Oxytropis, and Lupinus are not considered credible. They seem to be based upon visits of adult bruchids to flowers or extrafloral nectaries, upon erroneous identification of seeds affected, or upon mistaken interpretations of the literature.

The tribe Vicieae includes the genera Vicia, Lathyrus, Lentilla (or Lens), Pisum, Cicer, and Abrus. Cicer (which includes *C. arietinum* L., the chickpea, garbanzo, or gram) and Abrus (including *A. precatorius* L., the rosary-pea) are known to be infested by other Bruchidae, but no species of Bruchus has yet been recorded as affecting either genus. The other host genera all have some species affected; in some instances two or more genera may be attacked by one species of Bruchus.[5] These genera are very closely allied and merge into one another imperceptibly, particularly in the characters of seeds and pods, so that their separation is more or less conventional, being based on usage rather than on sound botanical differences. The seeds of Vicia, Lathyrus, and Pisum are often not easily distinguished as to species, or even as to genus, by botanists familiar with the plants. Hence, many records of host plants of species of Bruchus are indefinite from lack of specific determination of the plants affected or erroneous from misdetermination of the plant. Furthermore, the species of Bruchus are difficult to recognize and frequently have been determined wrongly. The records of attacks upon definite species of Vicieae are surprisingly few, and these few are less to be depended upon than is desirable.

Lentilla lens (L.) W. F. Wight (*Lens esculenta* Moensch), the lentil, is affected by *Bruchus lentis* Froelich, *B. ervi* Froelich, *B. signaticornis* Gyllenhal (*pallidicornis* Boheman), and *B. rufimanus* Boheman. The first two species are not known to affect other host plants. *B. signaticornis* is also found in the lentil-like seeds of *Vicia monanthos* Desfontaines. *B. rufimanus* seems to attack lentils only incidentally. The cultivation of lentils has been repeatedly abandoned in various parts of Europe on account of the attacks of one or another of the lentil bruchids. The writers have found no records of bruchids attacking the seeds of other species of Lentilla than those mentioned.

Pisum sativum L., the common pea with its varieties, is attacked by *Bruchus pisorum* (L.), *B. emarginatus* Allard, *B. tristiculus* Fahraeus, and perhaps by *B. tristis* Boheman. *B. emarginatus* seems to be the species that attacks the pea in India which has sometimes been determined as *B. affinis* Froelich. It is evidently quite as much a pea pest as the better known *B. pisorum*. *B. tristiculus* is more often found in seeds of Lathyrus. The writers have not been able to verify the records of *B. tristis* attacking the pea, and it is possible that they may be based on misdeterminations of *B. tristiculus*. Peyerimhoff (*10, p. 365*) has reported the pea weevil breeding in seeds of *P. elatius* Bieberstein, the reputed ancestor of the pea, in northern Africa, and the writers have found it in this species from Tiflis. The writers have discovered no records of the other species of Pisum being attacked by Bruchidae.

The species of the genus Lathyrus are not very familiar to laymen in the United States except *L. odoratus* L., the sweet pea. There is a considerable industry in the production of the seed of this species in California which is definitely threatened by the likelihood that *Bruchus affinis*, *B. tristis*, *B. tristiculus*, and *B. rufipes* Herbst[6] will

[5] It should not be inferred that species of Bruchus are the only Bruchidae affecting plants of the other genera of the tribe Vicieae. Certain species of Bruchidius and Callosobruchus affect a number of them. Some species of Bruchidius may be as definitely attached to some of them as the pea weevil is to the pea.

[6] Since this paragraph was written *Bruchus rufipes* has been intercepted by inspectors of the Bureau of Plant Quarantine in seeds of sweet pea (*Lathyrus odoratus*), common vetch (*Vicia sativa*), and hairy vetch (*V. villosa*), all of European origin.

be introduced in sweet peas or the seeds of other species of Lathyrus, particularly *L. sativus* L.[6] The grass pea (*L. sativus*) is an important food grain in the south of Europe and in India and is also imported into the United States for use as food. The Tangier pea (*L. tingitanus* L.) has been grown experimentally as a forage and green-manure plant, but it has not yet made a place for itself in our agriculture. Other species have also been grown as ornamentals, and still others experimentally as forage crops. *B. venustus* Fahraeus, *B. viciae* Olivier, *B. loti* Paykull, *B. rufipes*, *B. tristis*, *B. tristiculus*, *B. affinis*, and *B. atomarius* (L.) are more or less definitely known to breed in seeds of some species of Lathyrus. *B. altaicus* Fahraeus was described from the seeds of *L. tuberosus* L. from the Altai Mountains in central Asia, and the writers have recently determined as this species a bruchid found in the seeds of an undetermined species of Vicia from Turkestan.

Several species of Vicia are used for forage, hay, and green-manure plants, and the seeds of some of them are occasionally used for human food. *Vicia faba* L. (the broadbean) is a common food of man. *Bruchus rufimanus* seems to prefer *V. faba* as host and is principally known as an enemy of that plant. Peyerimhoff (*10, p. 365*) has recorded it as bred from *V. vestita* Boissier, which is a synonym of *V. lutea* L., in which the present writers have also found it breeding. The writers' notes also record it from *V. monanthos* Desfontaines, *V. sativa leucosperma* Moench, *V. narbonensis* L., and *Lentilla lens*. *B. dentipes* (Baudi) is reported from *V. faba*, and the writers have determined it from a wild Vicia from Haifa, Palestine. Bekman (*4*) records *B. dentipes* Baudi from *V. hyrcana* (=*hyrcanica* Fischer and Meyer). As before indicated, the writers have determined *B. signaticornis* from seeds of *V. monanthos* from Madrid, Spain. *B. viciae* Olivier, *B. atomarius* (L.), *B. ulicis* Mulsant and Rey, *B. rufipes* Herbst, *B. griseomaculatus* Gyllenhal, and *B. brachialis* Fahraeus are all recorded as breeding in seeds of species of Vicia.

COMMERCIAL CONDITIONS AFFECTING THE DISTRIBUTION OF BRUCHIDAE

The establishment of *Bruchus brachialis* in the eastern part of the United States is the result of a post-war speeding up of agricultural commerce which has increased the chances of introducing exotic Bruchidae into this country. Formerly commercial operations were so slow that seeds were not distributed in this country until a year or more after they were harvested. The bruchids in them therefore had time to emerge and if they were unable to reinfest the seed they died before they were scattered over the country. Within the last 20 years the principal shipping points of hairy-vetch seed imported into the United States have been changed from Baltic to Adriatic ports, where the earlier season makes it possible for the seed to be ready for market in the same season in which it is harvested. It is also much more certainly infested by *B. brachialis* in southern Europe than in the more northerly regions.

During the last three years several lots of living bruchids have been noticed in seeds imported under regular trade conditions, whereas in previous consignments the insects in the seeds examined were dead. *Bruchus brachialis* is the seventh species of Bruchidae known to have been accidentally established in continental United States.

[6] See note on page 740.

HISTORY OF BRUCHUS BRACHIALIS

Bruchus brachialis was described by Fahraeus (*13, p. 79*) from specimens sent to Schoenherr for description by Chevrolat, the material coming from Tours, France.

Mulsant and Rey (*8, p. 33*) described the male of *brachialis* as *pallidicornis* Schoenherr, and gave the first description of its distinctive sexual characters.

Allard (*1*) renamed *pallidicornis* Mulsant and Rey (not Gyllenhal) as *ruficornis*, and added Sicily to France as its habitat.

Perris (*9, p. 237*) records that in September, 1874:

> I fanned some wheat * * *. Under the fan the seeds of vetch and the *Bruchus* from them had accumulated in such numbers as to cover the ground. I noticed many *Bruchus pisi* [*pisorum*] and *granarius* [*atomarius*] with which I was not concerned, and persuaded myself the rest were *nubilus* [*rufipes*]. Entirely by chance, I picked up a few of them and saw that *nubilus* was in a very small minority and that the rest belonged to one or two species unfamiliar to me, though I might have mistaken them for *sertatus* [*ervi*] or *pallidicornis* [*signaticornis*], if I did not know that those species live in lentils. To have some for study when not too much pressed for time I collected some hundreds of them and found the two species to be *brachialis* Fahr. and *ruficornis* All., which according to Mr. Allard himself is only the male of *brachialis*.
>
> Here then is an insect which, judged by catalogues of rich collections, which I had run through recently, my colleagues do not have, which I did not myself possess, and of which, doubtless, I may take thousands more, for there are always, to nourish them, enough vetches among the wheat.

Baudi (*2, p. 13, 16*) described the species, placed it in Mylabris, and extended its range to include "all Italy and its Islands, Dalmatia, France, Spain, and Algeria."

Bedel (*3, p. 357*) placed it in Laria, gave its range in France as south of the Loire River, and added the record of its occurrence in Asia Minor.

Marchal (*7*) gives an account so pertinent to the present occasion that it is quoted in its entirety. Now, after nearly 30 years, his prediction of its establishment in this country has been fulfilled. The account is as follows:

> Perris has already indicated the presence of *Laria brachialis* Fahraeus in the seeds of vetches growing in fields of wheat. He found it there in quantity, associated with some other species, in seeds of Vicia accumulated under a fan which had just been used for cleaning wheat. The specific name of the plant was not definitely indicated by the author.
>
> Examination of a package received through Mr. Hariot, preparator at the Museum, results in finding this insect attacking the seeds of vetches cultivated as forage plants and notably those of *Vicia villosa*. The seeds of this plant sent to me were strongly infested by *Laria brachialis*, each seed attacked containing one of these insects.
>
> It should be noted that *Vicia villosa* is a plant of northern, eastern, and central Europe and does not grow wild in France. It has, however, for some years been cultivated frequently as a forage plant. On the other hand, *Laria brachialis* is a species belonging in the south of Europe and [according to Bedel] does not reach above the Loire in France except accidently. Apparently, then, it is only recently that this insect has adapted itself to *Vicia villosa*. [This inference may be doubted.]
>
> Not only in France is this plant menaced by this insect, which like all bruchids attacking plants under cultivation doubtless will not delay becoming cosmopolitan. The seeds sent to me had, indeed, been supplied by a French seed house and forwarded to Canada, whence they had been returned to France for examination of the insects contained in them. The approaching naturalization of *Laria brachialis* in America is an event to be foreseen.

Schilsky (*6, No. 29*) placed the species in Bruchus as restricted by himself and gave the most recent and most careful description of the species, a translation of which follows:

Bruchus brachialis Fahraeus. Ovate, black, covered with sparse fuscous pubescence beneath, sides of breast and venter whitish-maculate; pronotum and elytra whitish-variegate; pygidium covered with whitish-cinereous pubescence; prothorax almost twice as broad as long, sides parallel behind, dentate in the middle, hind angles rectangular; elytra short; front legs rufotestaceous, hind femora strongly dentate, hind tibiae ending in two spines at apex beneath. Length, 3–3.5 mm.

Male. Antennae rufotestaceous, joints 6 to 10 depressed, strongly transverse; front tibiae stout, compressed, slightly arcuate; middle tibiae curved, armed at apex with a truncate mucro.

Female. Antennae a little less depressed, black, first four or five joints and the last (and the front legs also) rufotestaceous; front femora black at base; front tibiae linear.

Variety a [male] with antennae infuscate in the middle; variety b [male], front femora black; variety c [female], front femora and last joint of antennae black; variety d [female], two joints of antennae rufous or ferruginous; variety e [female], antennae rufotestaceous, joints 6–7 or 6–9 infuscate; variety f [female], antennae bright rufotestaceous (Erivan); variety g [female], last joint of antennae black, middle tarsi rufescent. Nyons (from Baudi).

[Bibliography omitted.]

Antennae differing in color in the sexes as in *Br. pallidicornis;* front tibiae of male in both species widened but the fasciate markings of elytra lacking [in *brachialis*]; middle tibiae and tarsi black, the middle tibiae of males have very different characters. Body black, oval, similar to *atomarius*, sparse very short pubescence beneath, sides of breast and sternites with usual brighter maculae, brownish and dirty white variegate above, indefinite white markings of elytra not distinctly transverse-fasciate; scutellum and a macula in front of it whitish pubescent; pygidium evenly cinereous, generally with two small indistinct spots; pronotum almost twice as broad as long, densely rugosely punctured, the sides parallel behind, a small denticle in the middle, hind angles right angles, sides not emarginate in front of the angles; elytra short, oval, the striae fine, almost impunctate; hind femora strongly toothed, hind tibiae within with two equal very short spinules at apex.

Male. Antennae yellow, rarely infuscate in the middle (variety a), compressed, joints 2 to 4 fully as long as broad, or shorter, 5 considerably broader, the following joints very strongly transverse, emarginate at apex; front legs reddish yellow, their tibiae broad, compressed, parallel-sided, hence not arcuately expanded on the outer side [as t is in *signaticornis*, the other common species with broad front tibiae in the male]; middle tibiae curved downward, with a black, truncate plate at apex directed backward. In one male from Dalmatia (von Heyden collection) the middle tarsi are red.

Female. Antennae narrower, black; the 4 or 5 basal joints and the last, rarely the last two (variety d), reddish yellow; rarely [are] the antennae reddish yellow infuscate in the middle (variety e); base of the yellow front femora generally black to the middle, rarely entirely black (variety b); in some cases the last joint of the antennae is also black (variety c).

In central and southern Europe, in Asia Minor (Erivan, Anatolia), in Algeria.

SYNOPTIC TABLE OF THE NEAREST ALLIES OF BRUCHUS BRACHIALIS

According to Schilsky (*6, No. 12*), *Bruchus hamatus* Miller (*12, p. 228*) from Greece and Asia Minor is very closely allied to *B. brachialis*, but the pronotum is much less transverse and the pale dots on the elytra are arranged in two indistinct fasciae. The male *hamatus* has the secondary sexual characters of *brachialis*, but the fifth joint of the antennae is produced into a tooth or spine such that if the following joints were modified in the same manner the antennae would be pectinate. This is a rare species and its food plants are unknown.

Bruchus venustus Fahraeus (*13, p. 75*) is also closely allied to *brachialis*, but it has the hind femora with a small subbasal denticle beneath instead of the usual subapical tooth. It is found in the Caucasus, Hungary, and southern France and is considered a rare species.

Bruchus terminatus Wollaston (*14, p. 381*) from Teneriffe is apparently near *brachialis*, but it is more elongate and has more pale pubescence. The description of the subbifid spine at the apex of the middle tibiae would seem to refer to a truncate lamella such as occurs in *brachialis, venustus, ulicis, hamatus,* and *signaticornis*. The following outline should differentiate the males of this group:

1. Middle tibiae with acute tooth above apical lamella, front tibiae incrassate, antennae usually pale ..... *signaticornis*
   Middle tibiae without tooth above apical lamella 2
2. Front tibiae incrassate 3
   Front tibiae simple, antennae dark except at base ..... *ulicis*
3. Antennae pale, joint 5 produced into a tooth, middle femora triangularly widened, front legs only pale ..... *hamatus*
   Joint 5 of antennae normal 4
4. Hind femora with small denticle near base, without subapical tooth, front legs only pale, antennae with joints 1 to 8 pale, 9 to 11 dark ..... *venustus*
   Hind femora normal, with subapical tooth 5
5. Antennae pale, front legs only pale ..... *brachialis*
   Antennae dark, front legs and middle tarsi pale ..... *terminatus*

It is not possible at present to differentiate the females of these species or either sex of some other species which are as yet imperfectly known.

The hairy-vetch bruchid may be distinguished readily from the other two species of Bruchus now known to be established in the United States by its smaller size, much more transverse pronotum, greatly reduced pale pubescence, particularly on the elytra, and the coloration of the antennae, pale in the male, basal joints and terminal joint pale in the female. These three species may be distinguished from other North American Bruchidae by the emarginate lateral margin of the pronotum with a small distinct tooth before the emargination.

BRUCHUS BRACHIALIS IN THE NATIONAL MUSEUM

Bruchus brachialis has been recorded from no host plant other than *Vicia villosa* Roth. In addition to recently collected material from the eastern part of the United States, it is represented in the National Museum by three lots of adults with which are preserved seeds of *V. villosa* from which they had emerged. The first is from the Chittenden collection and was secured from the French exhibit in the Paris Exposition of 1889. The second was secured by the senior author in December, 1921, from seeds of *V. villosa* imported by a seed firm in Baltimore. Finding the seeds infested, this firm had brought the matter to the attention of the United States Department of Agriculture. The beetles contained in this lot were all dead when examined. The seeds were very lightly infested, much less than 1 per cent showing the work of *B. brachialis*. The third lot was submitted to the senior writer in September, 1930, by the manager of a seed-testing laboratory in Washington, D. C. The insects had been found living in samples of *V. villosa* seed submitted for testing by a Hungarian seed firm which proposed marketing the seeds in the United States. These

samples also showed less than 1 per cent infestation. After all the emerged bruchids had been removed, the seeds were kept under rigorous quarantine conditions at room temperature through the following winter, but no additional adults emerged. This suggests that at room temperature all the larvae transform and emerge in the fall or die before spring.

LIFE HISTORY OF BRUCHUS BRACHIALIS UNDER CONDITIONS PREVAILING IN NEW JERSEY

Adults of *Bruchus brachialis* were taken by sweeping in a fallow field near Haddon Heights, N. J., on June 12, 1931. On June 17, this field was again visited and many adults were collected. At this time eggs were being deposited in large numbers on the pods of the hairy vetch. Some preliminary observations made by the junior writer on the habits of the insect in this and in other patches of vetch are given here.

THE EGG

The egg is about 0.6 mm long, 0.2 mm wide at the widest point, which is near the anterior end, and 0.2 mm high. Viewed from above, it is more or less oblong in outline, with the ends rounded and the sides straight, or nearly so, and converging somewhat toward the posterior end. The outline is similar when viewed from the side, and from the end it appears nearly circular. The egg is glued lengthwise to the outer surface of the pod. The exposed surface is finely wrinkled.

The freshly deposited egg is a pale yellowish green. The hatched egg appears whitish or straw colored, owing to the débris with which the shell is filled by the larva in gaining entrance into the pod. In the field many of the eggs darken.

Eggs are deposited on the immature pod after it has attained a width of about one-fourth inch. Most of the eggs are placed near and parallel to the margins of the pod, with the posterior ends directed toward the apex of the latter. Twenty-five or more eggs were seen attached to a single pod, but the usual number was between 3 and 10. With the exception of those developed from late flowers, few pods escaped attack.

THE LARVA

The young larva gains entrance to its host by gnawing a hole through the broader end of the chorion of the egg and through the thin valve of the legume to which it is attached. It then turns toward the center of the pod in search of a seed. A few larvae do not find the seeds and therefore die within the pod. The larva may enter the seed at any point, the hilum being often preferred. In the ripe seed the entrance hole of the larva may be seen under low magnification as a small circular hole filled with frass. As many as seven entrance holes have been noted in a single seed, but rarely does more than one larva develop.

Once inside the seed, the larva develops rapidly, feeding upon the seed content. During growth the body of the larva is surrounded by dry frass, and when full grown it packs this loose material against the sides of the cavity made in feeding, to form the oval pupal chamber in which it is to transform. The frass is held in place by a thin cement substance, which upon drying hardens into a fibrous cocoon

very resistant to solvents. The full-grown larva often narrowly thins the margin of the circular area of the outer seed coat which forms the cephalic end of the cell. From the outside this thinned area appears as a narrow ring of pale olive green and is rather conspicuous against the darker color of the seed. The ring marks the area or "cap" which the adult cuts out in emerging. The middle of the cap is often thinned, giving it the lighter color.

The full-grown larva is a distinct yellow in color, and in outline resembles the larva of the common bean weevil (*Mylabris*) *Acanthoscelides obtectus* (Say) (*5*). After the larva has finished feeding and has the inside of the cap properly thinned and the cell lined, it is ready to pupate. Before pupating, however, it passes through a prepupal stage during which the shape of the pupa is gradually assumed, the body becoming less curved with a distinct constriction at the neck. This change takes place in about two days.

THE PUPA

The pupal stage lasts from 5 to 5½ days. During the first 3 days there is no change in the pale yellow color except in the eyes. Soon after pupation minute reddish spots appear along the hind margin of the eye. As these spots enlarge and darken, others gradually appear toward the front of the eye, the apex of the upper lobe being the last area to show the coloring. Each spot occupies the center of a facet, the interspaces being the pale body color until the fourth day, when the eye has become a uniform dark reddish brown. About this time a curved brownish line inside the hind coxal cavity appears and the mandibles start to become brown at the apex. The fifth day brings considerable change in color, the tarsal claws becoming fuscous at the base, and the body, except the abdomen, becoming various shades of pale reddish brown, the color being darkest at the articulations of the legs.

THE ADULT

The adult at first resembles the last stage of the pupa, but in less than 12 hours it has become normal in color. The wings remain exposed beyond the apex of the elytra for a day or two. The abdomen is much distended at first and about four days are required for it to contract to normal form. The adult is then thoroughly hardened and quite active.

Soon after it is fully hardened the adult cuts around the circular cap, pushes it away, and leaves the seed. The exit hole is located opposite to or near one end of the seed scar, but does not include any part of the scar. The first emergence from caged material collected June 17 took place about July 15, and during the rest of July adults emerged in enormous numbers from all collections. In the field on July 22 many emergences had already taken place and adults were numerous in unopened pods. The adult is unable to cut its way through the valve of the pod and depends upon the dehiscence of the pod for its escape.

Late pods in condition for oviposition on July 22 were without eggs and no recently deposited eggs were seen on any pods, indicating that the egg-laying season of the overwintering females had been ended for some time and that the newly emerged females were not depositing eggs.

The life history of *Bruchus brachialis*, therefore, may be briefly stated as follows: The overwintering females deposit eggs on immature pods of vetch in June, and the new generation of adults emerges from the seeds during the latter half of July and the first part of August but does not deposit eggs until the following June. It has not been ascertained that any individuals overwinter in the seeds.

QUANTITY OF FOOD CONSUMED AND EXTENT OF INFESTATION

As the larva does not migrate from one seed to another, the quantity of food consumed depends largely upon the size of the seed. This often directly affects the size of the adult, for small, poorly developed seeds produced adults 2 mm in length while the normal individual is 3 to 3.5 mm long. The entire content of the smaller seed is eaten, but the larger seed contains more food than is required by the larva. It is unlikely that a seed in which a larva has developed would be able to germinate.

A small representative sample of hairy-vetch seed from the first pods to ripen at Haddon Heights, N. J., was examined and the infestation noted. Of 785 seeds, 15 were not at all developed and are not considered. Of the remaining 770 seeds, 591 (76.7 per cent) produced adults or had pupae or full-grown larvae when opened, 32 (4.2 per cent) contained larvae that had died soon after entering the seed, and 147 (19.1 per cent) were not infested. This gives a total infestation of 80.9 per cent. One hundred of the uninfested seeds weighed 2.1 grams. Thus 1 pound would contain about 21,600 seeds, which would theoretically produce more than 17,000 bruchids.

PARASITES

The common bruchid egg parasite, *Uscana semifumipennis* Girault, was not noted to attack the eggs of *Bruchus brachialis*, but it should be expected to do so.

Six species of native American Chalcidoidea usually affecting coleopterous larvae and pupae in similar habitats were found parasitizing *Bruchus brachialis* in *Vicia villosa* at Haddon Heights late in July and in August, 1931. These species, kindly determined for the writers by A. B. Gahan, of the Bureau of Entomology, are *Eupelmus cyaniceps amicus* Gir., *Eupelminus saltator* (Lindemann), *Microdontomerus anthonomi* (Crawford), *Zatropis incertus* (Ashm.), *Habrocytus* sp., and *Eurytoma tylodermatis* Ashm. These species, except *E. saltator*, have been previously recorded as enemies of Bruchidae, but, as suggested, they are not in any peculiar sense bruchid parasites.

PRESENT DISTRIBUTION IN THE UNITED STATES

Bruchus brachialis is thus far known from five States—New Jersey, Delaware, Maryland, North Carolina, and Virginia. Numerous infestations in New Jersey were found, involving Camden, Burlington, Atlantic, and Cape May Counties, all in the southern part of the State. The junior writer reared numbers of the bruchid from vetch pods collected by D. P. Perry at Felton, Kent County, Del., July 1, 1931, and at Salisbury, Wicomico County, on the Eastern Shore of Maryland July 15, 1931. In September, 1931, a small sample of vetch seed was received at Haddon Heights, N. J., for experimental planting from

Roland McKee, of the Bureau of Plant Industry, United States Department of Agriculture. This lot of seed originated in the vicinity of Woodleaf, Rowan County, in the western part of North Carolina, where the smooth variety has been grown for several years. Several dead adults of *B. brachialis* were taken from seeds in this lot and a number of seeds with emergence holes were also obtained, showing that the insect is established in that State. During June, 1932, the junior author found this species in additional localities in Maryland, in the District of Columbia, and in adjacent Virginia.[7]

HOST PLANTS OF BRUCHUS BRACHIALIS

Vicia villosa is the only previously recorded host of *Bruchus brachialis* and is the species involved in the foregoing records. At Haddon Heights, Camden County, N. J., *V. cracca* L. was found to be lightly infested with this species of Bruchus. Normal emergence from the seeds was obtained during the first part of August from material collected July 11 and 23, 1931. The habits of the bruchid in these hosts, so far as known, are the same as in *V. villosa*.

During 1932 the junior author found *Bruchus brachialis* attacking the woolly-pot vetch (*Vicia dasycarpa* Ten.) and the Hungarian vetch (*V. pannonica* Crantz) in experimental plantings. *Vicia angustifolia* Roth growing abundantly in a field with *V. villosa* was not found to be infested.

ECONOMIC RELATIONS OF BRUCHUS BRACHIALIS

The hairy vetch (*Vicia villosa*) is an exceedingly adaptable forage and green-manure plant highly recommended by the United States Department of Agriculture for cultivation in this country in sections not favorable for the cultivation of alfalfa, red clover, or crimson clover. Its cultivation has been gradually increasing, particularly on the Atlantic seaboard from Washington south. The planting requirements in recent years have called for about 2,000,000 pounds of seed, of which about one half has been produced in this country and the remainder imported from Europe.

Vicia cracca is a circumpolar species extending from Europe across northern Asia and America to the Northeastern States, growing wild in sections north of those adapted to hairy vetch. It has not been found sufficiently useful in cultivation to compete with red clover and is of little commercial importance although it has most of the good qualities of hairy vetch. There is no production of its seed for commercial purposes. Hungarian vetch (*V. pannonica*) serves the same uses as the hairy vetch and the common vetch and recently has partly

[7] Since this paper was submitted for publication, notes have been received from G. W. Underhill, associate entomologist of the Virginia Agricultural Experiment Station, on the oviposition of *Bruchus brachialis* on the pods of hairy vetch near Richmond, Va., in 1931 and 1932. On 40 pods examined in late July, 1931, an average of 26 eggs per pod were found, with a maximum of 48 and a minimum of 11. The first emergence from the seed was noted on July 27, and a weevil emerged from almost every seed. Freshly laid eggs were observed on May 23, 1932, and were abundant in early June. Larvae hatched from the eggs of May 23 on May 27 and 28. In neither year were adults observed to cut their way out of the pods. A vetch not identified by Underhill, but certainly *Vicia angustifolia*, was abundantly associated with *V. villosa* but was not attacked by *B. brachialis*. Underhill believes that he found *B. brachialis* in small numbers in 1930 at Richmond, and the junior author certainly did at Haddon Heights, N. J. It is probable that the infestations in both places are recent. In New Jersey, at Richmond, Va., and in North Carolina *B. brachialis* was noticeably more abundant in 1932 than in 1931. Underhill's notes indicate that the season for *B. brachialis* at Richmond is three weeks or more earlier than at Haddon Heights. A thorough survey of the eastern part of the United States may show the bruchid to be established elsewhere.

superseded *V. sativa* in the vetch-growing sections of Oregon, where there is now a considerable commercial production of its seed.

The woolly-pod vetch (*Vicia dasycarpa*) seems to serve the same uses, but has not become as popular as some of the other vetches, and there is little or no commercial production of its seed in this country. *Bruchus brachialis* is important only in its relation to the production of the seeds of the vetches attacked by it. To what extent it will affect the production of hairy-vetch seed in Oregon, Indiana, Michigan, Ohio, New York, and the Carolinas, and of Hungarian-vetch seed in Oregon can not be predicted, but the North Carolina growers are already aware of increasing losses from it.

Whether the present species will also affect the common vetch (*Vicia sativa*) is entirely problematic. While no records have been encountered of attacks on other species of vetch, this must be considered of little significance until comprehensive studies have been made on the biology of this and other species of Bruchus. Several other species of Bruchus, however, attack *V. sativa* in Europe.

TWO OTHER SPECIES OF BRUCHUS FROM VETCHES

The bitter vetch (*Vicia ervilia* (L.) Willdenow) is another green-manure plant from the Old World occasionally cultivated experimentally in the United States, but it is still uncertain whether it will find an important place in American agriculture. Under certain commercial conditions the seeds of this species are imported and ground for mixed poultry feeds. The National Museum contains about 10 lots of *Bruchus ulicis* with notes indicating that the beetles were bred from seed of *V. ervilia* of European origin. Whether this insect affects other species of vetches is not a matter of record.

A sample of the seed of *Vicia cracca* introduced from Japan under the name *V. gemella* Crantz by the Office of Foreign Plant Introduction in 1903 was recently examined and a single female Bruchus found in it which agrees with the description of *Bruchus maculatipes* Pic (*11*). This vetch is so similar to *V. villosa* that it is almost certain that this species would be able to utilize the hairy vetch.

In May, 1932, individuals of the same species were sent by S. I. Kuwana to the Bureau of Entomology for determination, having been taken from among seeds in storage at Kyoto, Japan. Two males among these make it possible to recognize its close relationship with *Bruchus rufipes*, the main difference in *B. maculatipes* consisting in the almost complete suppression of the pale pubescent pattern of the pronotum and elytra.

SUMMARY

The hairy-vetch bruchid (*Bruchus brachialis* Fahraeus) is now established in the United States. In 1932 it was known to occur in New Jersey, Delaware, Maryland, the District of Columbia, Virginia, and North Carolina. It affects the seeds of the cultivated forage plants hairy vetch, woolly-pod vetch, Hungarian vetch, and the wild *Vicia cracca*. It is of economic significance because it destroys the seeds of its host plants.

The insect belongs to the restricted genus Bruchus. All the members of this genus deposit their eggs upon the green pods of the host plants. Their larvae feed within the seeds and transform there.

The adults emerge from the ripe seeds and can not reinfest them. *Bruchus brachialis* seems always to transform and leave the seeds in summer and hibernate elsewhere as an adult.

The adult is described and characters are pointed out to distinguish *Bruchus brachialis* from the closely allied European species of Bruchus and from all other American Bruchidae.

Notes on the life history and habits of *Bruchus brachialis* in New Jersey are recorded. In Haddon Heights, N. J., about 80 per cent of the seeds of volunteer plants of the hairy vetch produced during the egg-laying period of the bruchid were destroyed by it. Six species of native American Chalcidoidea were found parasitizing it.

The incidence of the different species of Bruchus upon seeds of the leguminous tribe Vicieae, including numerous important economic plants, is summarized, the records being based mainly upon interceptions made in the course of the work of the Department of Agriculture. Changed commercial conditions in handling these seeds have led to the establishment of *Bruchus brachialis* and make probable the establishment in the United States of other destructive species affecting plants upon which agricultural industries are based.

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THE ASSOCIATIVE EFFECTS OF FEEDS IN RELATION TO THE UTILIZATION OF FEED ENERGY[1]

By E. B. FORBES, *Director*, WINFRED W. BRAMAN and MAX KRISS, *Associate Professors of Animal Nutrition*, and R. W. SWIFT, *Associate in Animal Nutrition*, with the collaboration of ALEX BLACK, DONALD E. FREAR, O. J. KAHLENBERG, F. J. MCCLURE, and LEROY VORIS, *Institute of Animal Nutrition, Pennsylvania State College*

INTRODUCTION

The question of methods of measurement of the net energy of single feeding stuffs and of rations has been before this Institute, in one form or another, for many years, either as such values have been directly sought, or as efforts have been made to analyze the problem and to elucidate the underlying principles involved.

In connection with the direct approach to this subject consideration has been given to various methods of determining net energy (*11*)[2] and to a new method of applying the net energy conception in the derivation of a feeding standard for milk production (*14*). In the analysis of the fundamental aspect of this problem studies have been made on the influence of type and age on the utilization of feed (*2*); on the influence of position, as to standing or lying, on metabolism (*3*, *17*); on the influence of the degree of fatness on the utilization of feed (*5*); on the relative utilization of feed energy for maintenance, body increase, and milk production (*13*, *18*); on the influence of environmental temperature on the heat production (*9*); on the energy metabolism in relation to the plane of nutrition (*10*, *11*); on the analysis of the curve of heat production (*15*, *16*); and on the balance of nutrients, that is, the supplementing effects of feeding stuffs in combination, as affecting the utilization of feed energy (*7*, *12*).

Among the various feeding stuffs investigated at this institute corn (maize) meal has received relatively much attention, and the methods employed in measuring its net energy embrace the various procedures and problems involved in this estimation as applied to feeding stuffs in general, but especially to feeds of concentrated character.

The general point of view expressed by these methods and the earlier of the detailed procedures originated with Armsby. During the decade since his death these procedures have been continuously studied and frequently modified, with the hope of improvement, since no method for determining the net energy of individual feeding stuffs had, or has, been found that can be relied upon for concordant and defensible results.

The first attempt to determine the net energy value of corn meal was made by Armsby and Fries in the second of their respiration-calorimetric studies, in 1902 (*1*). The results obtained with a steer on a basal ration of red-clover hay were compared with the results obtained when to this basal ration corn meal in different quantities

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[2] Reference is made by number (italic) to Literature Cited p. 769.

was added, in two experimental periods. This procedure is usually referred to in recent discussion as the "feed-and-plane-difference method," since there was a difference not only of kind but also of quantity in the rations compared. The values obtained for corn meal in this experiment were widely divergent, and only one of them could be considered as plausible.

Subsequently, Armsby and Fries repeated the determination of the net energy value of corn meal in two series of experiments. In one of these (*6*) corn meal was combined with red-clover hay, and in the other (*4*) mixed hay was used as the roughage. In both of these series of experiments, however, the net energy was determined on the mixed ration by feeding it at two planes of intake; the net energy of the roughage was determined by feeding alone, at two planes of intake; and the net energy of the corn was computed by a difference process depending on the assumption that the value of the roughage in the mixed ration was the same as when fed alone. This procedure has been shown to have certain advantages (*19*) over the earlier one, notably in that there is likely to be less exaggeration of experimental errors. It disregards, however, the influence of the plane of nutrition on the utilization of feed energy, which has been shown to be an important factor in this relation (*10, 11*).

The values obtained for corn meal in these experiments were diverse and thus could not be considered reliable.

Since the adoption of the directly observed fasting katabolism of cattle as a measure of the maintenance requirement of energy, and as a base value in energy metabolism, the writers have endeavored in several series of experiments (*10, 11*) to determine the net energy value of corn meal both for maintenance and for body increase, employing procedures that give full recognition to the factor of plane of nutrition. The essential feature of one of these procedures, the "heat-increment-proportional method," is the assumption that the heat-increment value of an individual feeding stuff for maintenance is to its heat-increment value for body increase as the heat-increment value of a mixed ration, of which it is a component, for maintenance, is to the heat-increment value of the same ration for body increase.

Another procedure, the "net-proportional method," assumes that the net energy value of a single feeding stuff for maintenance is to its net energy value for body increase as the net energy of a mixed ration, of which it is a component, for maintenance, is to the net energy of the mixed ration for body increase.

Neither of the above-mentioned methods yielded consistent net energy values of single feeding stuffs for body increase. In fact, the results obtained strongly suggested associative effects of the feeding stuffs fed in combination, on the utilization of their energy, in addition to the influence of differences in the plane of nutrition of the rations compared.

The first direct demonstration of such associative effects of feeding stuffs was obtained by the writers in subsequent experiments (*12*) in which corn meal was fed exclusively and in combination with alfalfa hay, at a maintenance level. The heat increment of the corn meal when fed alone was found to be considerably greater (about three times) than that computed for it from results obtained when the corn meal was fed combined with the hay, while the metab-

olizable energy value of the corn meal was practically the same when it was fed alone as when fed with hay.

In the present investigation corn meal was added, in different experimental periods, to three basal maintenance rations differing radically in composition, thus providing for the further study of the early, simple, and direct feed-and-plane-difference method of Armsby for determining net energy values of individual feeding stuffs, and also for an investigation of the fundamental problem of associative or supplementing effects of feeds in combination.

The mixed rations resulting from the combination of corn meal with the basal maintenance rations used are unquestionably better balanced, nutritively, than is corn meal alone, and the effects of the combination are logically reciprocal; but inasmuch as there is no scientific method for apportioning these effects, the differentation of the values of the basal ration and the supplementary corn meal is possible only if it be assumed that one of these two ration components is the active agent and the other is acted upon.

In the light of general knowledge the three basal rations employed were considered to be of poor, of low-medium, and of excellent nutritive value. The net energy value given to the corn meal by the supplementing effect of the combination of corn meal and these basal rations would obviously be in accord with the effectiveness of the reciprocal supplementation, and not necessarily in accord with the nutritive balance of the basal rations.

In other words, an unbalanced basal ration fed with corn meal might give to the corn meal a higher net energy value, if the basal ration were unbalanced in a way to balance the corn meal, than would a perfectly balanced basal ration fed with corn meal.

Theoretically, at least, any "effective" deficiency of any essential nutrient must eventually affect the utilization of the energy of the food, and the question whether a specific nutritive deficiency is effective at a particular time obviously depends on (1) the nature of the deficiency, (2) the extent of the requirement of the animal for the deficient nutrient, (3) the extent and availability of the body reserve of the nutrient which is deficient in the ration, and (4) the length of the period of experimentation.

Thus, since corn meal is characterized by a high metabolizable energy value, but by marked nutritive deficiencies, as previously noted, a question of importance is whether, in the course of a 28-day feeding period, these deficiencies affect the utilization of the energy of the corn meal in an unfavorable manner, or whether the animal that has eaten the corn meal is either partly or wholly protected from these deficiencies, by drafts upon the nutritive reserves of its body, so that the deficiencies are without apparent or immediate effect on the net energy value of the corn meal.

In this relation it would be exceedingly important to know how quickly and to what extent specific nutritive deficiencies affect the utilization of the energy of feeding stuffs. For instance, a deficiency of protein in a ration might conceivably affect the utilization of its energy within one day, a deficiency of vitamin B within a week, a deficiency of vitamin A within a month, a deficiency of phosphorus within 6 months, and a deficiency of vitamin D not at all, while the food consumption remains unaffected.

Also, an inorganic compound, calcium phosphate, for instance, or a vitamin in an experiment of adequate length might "earn" a net energy value if added to a ration which is deficient in the substance but would have no energy value in other relations.

Furthermore, the senior writer learned many years ago that in feeding for body increase the smaller the proportion in which a protein supplement to corn is employed the greater will be its apparent value, per unit of itself.

From the foregoing it will be understood that in the authors' opinion much remains to be learned as to the effects of specific kinds and degrees of nutritive deficiency upon net energy values, and as to the time element in such relations. Also, conceding that different net energy values will be found (as affected by drafts upon nutritive reserves of the animal body) in experimental periods of different length, there remains the question, What would be the most nearly true or characteristic value, or the one of greatest significance, as applying to particular conditions of feeding practice?

PLAN OF EXPERIMENTATION

A duplicate series of seven energy-balance experiments was conducted during the fall, winter, and spring of 1931–32, with two 2-year-old steers, as follows: (1) During fast; (2) at the maintenance level, with three rations of different character; and (3) at a production level, the rations being the same as the three fed at the maintenance level plus corn meal in quantities equivalent to one-half of the estimated maintenance requirement of energy.

The schedule of experimentation is given in Table 1, together with the daily rations, and the live weights of the experimental subjects.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals in experiment No. 261*

Period No.	Steer	Preliminary experimental feeding period	Periods of excreta collection	Calorimeter periods	Plane of nutrition	Rations fed daily							Average live weight of animals
						Oat straw	Timothy hay	Alfalfa hay	Corn meal	Linseed meal	Wheat bran	Molasses	
		Days	*Days*	*Days*		*Kg*	*Kg*	*Kg*	*Kg*	*Kg*	*Kg*	*Kg*	*Kg*
1	A		4	4	Fasting								385.3
2	B		4	4	do								318.8
3	A	4	12	3	Maintenance	2.500			1.800			0.180	393.5
4	B	8	21	3	do	2.290			1.640			.160	323.6
5	A	4	10	3	1½ maintenance	2.500			3.268			.180	409.1
6	B	4	10	3	do	2.290			2.982			.160	335.5
7	A	9	21	3	Maintenance		2.976		1.502				401.0
8	B	10	21	3	do		2.590		1.308				325.8
9	A	14	21	3	1½ maintenance		2.976		3.004				425.1
10	B	14	21	3	do		2.590		2.616				343.2
11	A	8	21	3	Maintenance			3.500		0.910	0.910		432.2
12	B	9	21	3	do			3.024		.786	.786		351.7
13	A	9	21	3	1½ maintenance			3.500	1.538	.910	.910		453.1
14	B	14	21	3	do			3.024	1.348	.786	.786		372.7

The three basal maintenance rations differed widely as to protein, mineral, and, presumably, as to vitamin contents. The first ration

consisted of oat straw and corn meal (1.4:1), supplemented with a small quantity of molasses to increase its palatability; the second consisted of timothy hay and corn meal (2:1); while the third was made up of alfalfa hay, linseed meal, and wheat bran in a proportion of 3.8:1:1.

Inasmuch as the maintenance rations supplied nutriment approximately as required to sustain energy equilibrium, the results obtained for the added corn meal, in the production periods, were comparable in that the plane of nutrition was essentially the same in all three cases.

The fasting experiments with the two animals provided a basis for determining the energy cost of maintenance, and of standing as compared with lying. This information was required in computing the heat production of the animals, standing or lying, in all experimental periods to a standard day, and to the basis of a uniform live weight.

In the fasting periods tne heat production and the gaseous exchange were measured continuously in the respiration calorimeter for four consecutive days, and the attainment of the status of true fast was indicated by the respiratory quotients, which were determined in intermittent samples of the air current taken every three hours.

The experimental periods during which the animals received feed consisted of a preliminary feeding period of 9 to 14 days, a digestion period ordinarily 21 days in length, and a calorimeter period to 3 days—usually the last 3 of the digestion period.

Each calorimeter period was preceded by a preliminary period of 14 hours, during which the experimental ensemble was established in adiabatic balance. The animal was inside the calorimeter during this time.

In three cases (periods 3, 5, and 6) the digestion periods as well as the preliminary periods were of necessity considerably shortened, as indicated in Table 1, because of unsatisfactory behavior of the experimental subjects.

During the digestion periods quantitative collections were made of feces and urine, while during the calorimeter periods, in addition to the collection of the visible excreta, the gaseous products and the heat production of the animals were measured.

In all the feeding periods the heat production was measured directly as usual, and checked by indirect determination from the carbon, nitrogen, and energy balances. Final comparisons and computations, in the utilization of the results, were based on the average of the direct and the indirect measurements.

EXPERIMENTAL SUBJECTS

The steers used as subjects in these experiments were designated A and B. Steer A was a crossbred Aberdeen-Angus-Shorthorn, born January 25, 1930, and steer B was a purebred Shorthorn, born April 16, 1930. Their average live weights in the first two experimental feeding periods (Nos. 3 and 4) were 393.5 kg and 323.6 kg, respectively.

EXPERIMENTAL DATA

The chemical composition and digestibility of the rations, and of three of the individual feeding stuffs, corn meal, oat straw, and timothy hay, are given in Table 2.

TABLE 2.—*Chemical composition and digestibility of rations, and of corn meal, oat straw, and timothy hay*

Period No.	Steer	Ration No. or feeding stuff	Chemical composition (dry basis)				Energy per gram	Digestion coefficients				
			Crude protein	Carbohydrate		Ether extract		Crude protein	Carbohydrate		Ether extract	Energy
				Nitrogen-free extract	Crude fiber				Nitrogen-free extract	Crude fiber		
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3	A	3	7.01	56.48	24.35	3.23	4,375	46.7	72.8	54.6	61.8	62.2
4	B	4	7.00	56.43	24.41	3.23	4,373	44.6	70.7	50.3	58.3	59.7
5	A	5	7.34	62.24	19.41	3.82	4,461	52.9	75.6	51.4	72.3	66.3
6	B	6	7.34	62.22	19.46	3.82	4,459	41.3	71.9	50.7	63.7	63.6
7	A	7	7.14	61.07	24.10	3.19	4,497	54.7	71.9	46.9	66.5	62.0
8	B	8	7.00	61.02	24.27	3.14	4,490	42.9	69.8	42.9	63.1	58.9
9	A	9	7.47	65.69	19.49	3.62	4,511	56.8	77.6	45.4	70.1	66.0
10	B	10	7.51	65.46	19.73	3.58	4,503	43.4	75.7	41.3	63.4	62.2
11	A	11	22.13	43.06	23.33	3.24	4,536	79.7	77.0	41.9	58.2	65.1
12	B	12	22.10	43.03	23.31	3.23	4,532	77.9	76.1	33.9	57.4	62.1
13	A	13	19.54	51.13	18.86	3.67	4,539	76.9	82.5	34.0	65.8	67.9
14	B	14	19.47	51.23	18.78	3.67	4,536	76.8	81.9	26.5	64.4	66.2
5	A	Corn meal	9.59	81.81	2.03	5.11	4,538	70.6	82.3	(−66.9)	92.3	79.5
9	A	do	9.59	81.81	2.03	5.11	4,538	62.7	91.5	(19.5)	77.4	78.8
13	A	do	9.94	81.13	2.20	5.28	4,551	54.0	93.2	(−241.8)	83.0	78.3
6	B	do	9.59	81.81	2.03	5.11	4,538	(32.1)	(74.8)	(62.5)	(73.9)	(76.0)
10	B	do	9.94	81.13	2.20	5.28	4,551	44.7	90.1	(19.0)	64.1	72.3
14	B	do	9.94	81.13	2.20	5.28	4,551	68.1	92.9	(−216.4)	79.9	80.6
3	A	Oat straw	5.13	43.45	41.91	2.56	4,483	(12.3)	(60.1)	(58.5)	(23.3)	(48.3)
5	A	do	5.14	43.00	42.50	2.53	4,490	(12.3)	(60.1)	(58.5)	(23.3)	(48.3)
4	B	do	5.13	43.45	41.91	2.56	4,483	(62.5)	(65.2)	(50.0)	(38.6)	(46.4)
6	B	do	5.14	43.00	42.50	2.53	4,490	(62.5)	(65.2)	(50.0)	(38.6)	(46.4)
7	A	Timothy hay	6.06	52.05	34.68	2.33	4,528	(48.0)	(57.2)	(47.2)	(55.1)	(54.1)
9	A	do	5.56	51.30	36.09	2.27	4,537	(48.0)	(57.2)	(47.2)	(55.1)	(54.1)
8	B	do	5.86	52.00	35.07	2.25	4,527	(41.4)	(54.5)	(43.1)	(61.8)	(52.6)
10	B	do	5.31	51.50	36.55	2.02	4,514	(41.4)	(54.5)	(43.1)	(61.8)	(52.6)

If the rations are designated by the experimental period numbers, rations 3, 4, 5, and 6, composed of corn meal, oat straw and molasses, may be characterized, in the light of general information, as unpalatable and nutritively deficient, especially because oat straw is a very poor roughage, although a great deal of it is eaten by cattle.

Rations 7, 8, 9, and 10, composed of corn meal and timothy hay, may be regarded as of low-medium quality. These rations, like those in the preceding group, are deficient in variety of components, and timothy hay is a second-rate roughage for cattle.

Rations 11, 12, 13, and 14 were of high grade. The roughage, alfalfa hay, was of the best quality, and the grain components were palatable, varied, and nutritious. The rations were rich in protein, calcium, and phosphorus and are not known to have been lacking in any essential nutrient.

In these three groups of rations, therefore, corn meal was fed in decidedly poor, in low-medium, and in excellent combinations.

The protein intake differed much from period to period but was, as intended, at all times safely above the maintenance requirement, as shown by the invariably positive nitrogen balances. (Table 4.) The nitrogen intake, in grams, per kilogram of live weight of steers A and B in the several experimental periods was as follows: Periods 3 and 4, 0.117 and 0.129; 5 and 6, 0.151 and 0.168; 7 and 8, 0.114 and

0.119; 9 and 10, 0.147 and 0.160; 11 and 12, 0.395 and 0.420; and 13 and 14, 0.424 and 0.447.

The ratios of carbon to nitrogen in the basal rations containing oat straw, timothy hay, and alfalfa hay, respectively, were 39 to 1, 41 to 1, and 13 to 1. In the supplemented rations (with corn meal) these ratios were 38 to 1, 39 to 1, and 15 to 1, respectively.

The energy of the digestible nutrients of rations 5 and 6 was greater than that of rations 3 and 4, on account of the added corn meal which the former contained. A similar difference prevailed, for the same reason, between rations 9 and 10 as compared with 7 and 8, and between rations 13 and 14 as compared with 11 and 12.

In each of the six cases in which the same ration was fed to the two subjects steer A digested an appreciably greater part of the energy-producing nutriment of the ration than did steer B, the differences, as shown in Table 2, being 2.5, 2.7, 3.1, 3.8, 3.0, and 1.7 per cent, respectively. Also, without exception, steer A digested all other kinds of nutriment in each ration more efficiently than did steer B.

This comparison, therefore, shows that individuality is a significant factor affecting digestible nutrient and therefore net energy values.

The digestibility of the corn meal by the two steers, as affected by the feeds with which it was fed, is given in Table 2. Inasmuch as the digestibility of the corn meal was of necessity computed by a difference or subtraction procedure, which served to relate and to express all errors of theory and of work as though affecting only the values of the comparatively small quantities of corn meal; and, further, inasmuch as the computation of the coefficients for corn meal depended on the assumption (which is not exactly true) that the digestibility of the corn meal and of the basal part of the ration in each case was the same when fed separately as when fed together, marked inconsistencies appear in these computed digestion coefficients. These inconsistencies are only the beginning of the troubles encountered in efforts to determine net energy values of individual feeding stuffs.

The first three of these values for corn meal were obtained, steer A being the subject, in periods 5, 9, and 13, while steer B was the subject in periods 6, 10, and 14. Manifestly unrepresentative data, or data which for any reason are considered questionable, are inclosed in parentheses. The unreliability of the data in period 6 was due to the fact that the animal did not take the feed satisfactorily, the collection period was short, and the correspondence between the ration and the feces was obviously imperfect.

In considering these six digestion coefficients for corn meal a comparison should be made of the performance of the two steers in periods 5 and 6, 9 and 10, and 13 and 14. In view of the foregoing observations, however, these digestion coefficients of corn meal can not be closely interpreted.

In other words, digestion coefficients of individual feeding stuffs, computed by difference, must be considered as conventional—especially because the digestibility of foodstuffs given individually may not be the same as when fed in combination and because there is no scientific method for apportioning the digestibility of a mixed ration among its component feeding stuffs.

The individual digestion coefficients of oat straw and timothy hay were also computed, by assuming that the values for corn meal,

representing the difference between two rations, apply also to the corn meal within these rations. The second difference process involved in this computation serves still further to magnify the errors incident to the first, and the values thus derived for the roughages are obviously without definite significance. The process of computation used is superficially logical, but, as explained, the effects of the errors of work and of fundamental assumption render the results quite imperfectly representative.

TABLE 3.—*Carbon dioxide, water vapor, methane, and heat production per day*

Period No.	Steer	Calorimeter day	CO_2	H_2O	CH_4	Heat by radiation and conduction [a]	Latent head of H_2O vapor	Total heat				
								Directly observed	Directly observed, corrected for body gain	Indirectly computed	Average, computed and observed	Corrected to standard day
			Grams	*Grams*	*Grams*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
		First	3,697.4	.....	109.2	5,210	.....	9,127	.....	.....	.....	.....
		Second	3,816.6	.....	113.9	5,510	.....	9,427	.....	.....	.....	.....
3	A	Third	3,816.9	.....	114.5	5,704	.....	9,621	.....	.....	.....	.....
		Average	3,777.0	6,684	112.5	5,475	3,917	9,392	9,275	9,176	9,226	9,298
		First	3,411.6	.....	106.0	5,234	.....	8,414	.....	.....	.....	.....
		Second	3,383.4	.....	103.1	5,324	.....	8,504	.....	.....	.....	.....
4	B	Third	3,414.2	.....	99.6	5,425	.....	8,605	.....	.....	.....	.....
		Average	3,403.1	5,426	102.9	5,328	3,180	8,508	8,509	8,285	8,397	8,632
		First	4,369.2	.....	139.8	7,969	.....	10,414	.....	.....	.....	.....
		Second	4,472.8	.....	140.9	7,936	.....	10,381	.....	.....	.....	.....
5	A	Third	4,410.5	.....	143.2	7,903	.....	10,348	.....	.....	.....	.....
		Average	4,417.5	4,165	141.3	7,936	2,445	10,381	10,333	10,138	10,236	10,538
		First	3,924.4	.....	[b]122.5	5,807	.....	9,182	.....	.....	.....	.....
		Second	3,992.4	.....	123.8	6,094	.....	9,469	.....	.....	.....	.....
6	B	Third	4,091.4	.....	122.5	6,298	.....	9,673	.....	.....	.....	.....
		Average	4,002.7	5,750	122.9	6,066	3,375	9,441	9,481	9,218	[c]9,481	9,645
		First	3,708.3	.....	111.1	6,973	.....	8,993	.....	.....	.....	.....
		Second	3,642.7	.....	110.9	6,915	.....	8,935	.....	.....	.....	.....
7	A	Third	3,632.4	.....	109.2	6,718	.....	8,732	.....	.....	.....	.....
		Average	3,661.1	3,440	110.4	6,869	2,020	8,887	8,862	8,768	8,815	9,015
		First	3,181.5	.....	93.5	5,688	.....	7,745	.....	.....	.....	.....
		Second	3,107.5	.....	89.9	5,478	.....	7,535	.....	.....	.....	.....
8	B	Third	3,122.9	.....	91.3	5,438	.....	7,495	.....	.....	.....	.....
		Average	3,137.3	3,504	91.6	5,535	2,057	7,592	7,618	7,685	7,652	7,985
		First	4,553.9	.....	144.9	6,862	.....	10,604	.....	.....	.....	.....
		Second	4,596.5	.....	143.8	7,053	.....	10,795	.....	.....	.....	.....
9	A	Third	4,630.5	.....	139.3	7,163	.....	10,905	.....	.....	.....	.....
		Average	4,593.6	6,374	142.7	7,026	3,742	10,768	10,698	10,669	10,684	10,885
		First	3,925.2	.....	111.2	6,321	.....	9,417	.....	.....	.....	.....
		Second	4,053.6	.....	113.5	6,593	.....	9,689	.....	.....	.....	.....
10	B	Third	3,957.1	.....	109.7	6,543	.....	9,639	.....	.....	.....	.....
		Average	3,978.6	5,275	111.5	6,486	3,096	9,582	9,577	9,403	9,490	9,637
		First	4,256.4	.....	118.0	7,137	.....	10,754	.....	.....	.....	.....
		Second	4,276.2	.....	120.6	6,940	.....	10,557	.....	.....	.....	.....
11	A	Third	4,188.0	.....	117.0	6,802	.....	10,419	.....	.....	.....	.....
		Average	4,240.2	6,162	118.5	6,960	3,617	10,577	10,504	10,371	10,438	10,598
		First	3,742.9	.....	98.2	6,632	.....	9,604	.....	.....	.....	.....
		Second	3,684.8	.....	96.9	6,142	.....	9,114	.....	.....	.....	.....
12	B	Third	3,752.0	.....	95.4	6,585	.....	9,557	.....	.....	.....	.....
		Average	3,726.6	5,063	96.8	6,453	2,972	9,425	9,488	9,375	9,432	9,511
13	A											
		First	4,483.1	.....	133.8	6,667	.....	10,586	.....	.....	.....	.....
		Second	4,606.0	.....	136.2	7,276	.....	11,195	.....	.....	.....	.....
14	B	Third	4,543.0	.....	139.4	6,860	.....	10,779	.....	.....	.....	.....
		Average	4,544.0	6,688	136.5	6,934	3,919	10,853	10,858	10,798	10,828	10,944

[a] Heat measured by water current diminished by heat liberated on condensation of water on absorbers.
[b] Based on one 12-hour subperiod.
[c] Observed heat only.

The data for carbon dioxide, water vapor, methane, and heat produced are given in Table 3, the heat production being computed,

finally, to the basis of the standard day of 12 hours standing and 12 hours lying, and to the basis of a standard live weight.

The data for gaseous outgo and heat production of steer A in period 13 are omitted, because they were vitiated by unfavorable experimental conditions. The difficulty apparently began with the steer's shedding hair into his urine funnel, which clogged the flow of urine and led to its overflowing onto the floor on which the steer stood or lay. For this reason the steer was uncomfortable and changed position (as to standing and lying) 62 per cent more times than did steer B on the same ration. He developed the habit of standing out of position with his forefeet up on the lid of the feed box. This disarranged the feces apron, and feces were spilled onto the floor.

The air in the calorimeter chamber became so strongly ammonical that the drip water from the copper heat absorbers was blue. The attendant had to enter the chamber seven times during the 3-day period to rearrange the harness and the stall appurtenances.

In these experiments the daily elimination of carbon dioxide, methane, and heat varied but little during the three days of each calorimetric measurement.

The rate of ventilation, however, was less than that which ordinarily prevailed in the experiments of previous years, and considerable quantities of water condensed on the absorber pipes. The water remaining in the air current was fairly constant in quantity; but that which was condensed on the absorbers, and consequently the total eliminated, appeared to vary much from day to day, since the flow of condensed water from this apparatus is not accurately quantitative for 24-hour periods. The quantities of this condensed water, as observed on the separate days of a 3-day calorimetric measurement, therefore, were averaged, as were also the accurately measured quantities of moisture carried from the calorimeter in the air stream, these two portions being added to give the quantity of the total outgo of water vapor.

TABLE 4.—*Intake and balance of matter and energy*

Period No.	Steer	Daily intake of—						Daily balances of—					
		Dry matter	Water	Nitrogen	Carbon	Gross energy	Metabolizable energy	Water	Nitrogen	Carbon of protein	Carbon of fat	Total carbon	Energy
		Grams	*Grams*	*Grams*	*Grams*	*Calories*	*Calories*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*
3	A	3,954	10,256	46.0	1,811	17,429	8,767	−5,792	8.4	26	−51	−25	−409
4	B	3,610	14,300	41.9	1,654	15,917	7,646	133	6.3	20	−65	−45	−639
5	A	5,103	11,208	61.6	2,366	22,899	12,659	−2,503	16.7	53	167	220	2,521
6	B	4,661	16,601	56.2	2,161	20,918	11,155	1,749	13.0	41	128	169	1,937
7	A	3,970	7,948	45.6	1,863	17,986	8,980	−1,230	5.6	18	5	23	212
8	B	3,428	11,487	38.7	1,605	15,528	7,410	1,314	4.1	13	−31	−18	−275
9	A	5,206	10,274	62.6	2,436	23,620	12,891	−3,504	19.0	60	138	198	2,222
10	B	4,530	16,133	54.8	2,122	20,532	10,656	−321	9.7	31	80	111	1,253
11	A	4,801	16,856	170.9	2,222	21,914	10,978	−3,601	16.2	51	14	65	607
12	B	4,148	22,158	147.7	1,920	18,934	9,025	3,102	12.0	38	−54	−16	−350
13	A	6,116	20,795	192.1	2,833	27,899	14,850	------	24.4	------	------	------	------
14	B	5,315	23,706	166.6	2,462	24,245	12,558	135	22.6	71	93	164	1,760

The intake and the balance of matter and energy are given in Table 4. It is of interest to note that the nitrogen balances for both animals were all positive and were of significant magnitude even in

the energy-maintenance periods on the low-protein rations consisting of oat straw, corn meal, and molasses (periods 3 and 4), and on those made up of timothy hay and corn meal (periods 7 and 8). The daily balances of energy in the maintenance periods, however, were comparatively small and show that the estimates of energy requirement employed in computation of the rations were satisfactory.

The quantities of nitrogen excreted in the urine during the first eight periods, when the animals were on the low-protein rations, are much less than as usually observed, because of the low protein intake and the sparing effect of carbohydrates on protein metabolism. When the animals were on the oat straw-corn meal rations (periods 3 to 6, inclusive) the daily urinary nitrogen was 13.8 g, 13.0 g, 16.5 g, and 10.9 g, respectively, which is equivalent to an average of 3.8 g per 100 kg of body weight for steer A, and to an average of 3.6 g for steer B; but when they received the timothy hay-corn meal rations (periods 7 to 10, inclusive) the daily urinary nitrogen was 19.3, 12.5, 16.6, and 14.1 g, respectively, which is equivalent to an average of 4.3 g per 100 kg of body weight for steer A, and an average of 4.0 g for steer B. In previous experiments (*10*) the average daily nitrogen excretion of four steers which received no feed was found to be 8.1 g, 9.0 g, 11.3 g, and 9.8 g, respectively, per 100 kg of body weight.

It is a thoroughly established fact that the urinary-nitrogen excretion during fast does not represent the minimum protein requirement of the animal, since during fast some protein is katabolized for energy production. Many workers have observed that on protein-free rations, or on rations very poor in protein, the protein katabolism is reduced to a level considerably lower than that which prevails during fast.

The data for urinary nitrogen during fast in this series of experiments are not reported, but during periods 3, 4, and 8 the nitrogen requirement, as measured by the digestible nitrogen minus the positive nitrogen balance (the remainder being equal to the urinary nitrogen) was 13.8, 13.0, and 12.5 g, respectively, which is equal to 3.5, 3.1, and 3.8 g per 100 kg of live weight, or to an average of 21.7 g of crude protein. This is less than half of the protein allowed for maintenance that is indicated by prevailing feeding standards for cattle.

The quantities of nitrogen excreted in the urine of the animals on the high-protein rations (periods 11 to 14, inclusive) are about 8 to 10 times as great as those when they were on the low-protein rations. The carbon-nitrogen ratios in the urine in periods 11 to 14 (high-protein rations) are 1.2, 1.2, 1.3, and 1.3, respectively, while the carbon-nitrogen ratios in the urine in periods 3 to 10 (low-protein rations) are 3.6, 3.2, 3.2, 3.7, 3.3, 3.8, 3.9, and 4.0, respectively. These observations indicate that under a given set of conditions the carbon-nitrogen ratio in the urine is fairly constant, but that there is no fixed relation between the carbon content of the urine and the quantities of protein katabolized, and that some of the carbon in the urine represents nonnitrogenous substance.

In computing the intake and the balance of matter and energy the energy of the urine and of the protein gained or lost was corrected, as usual, for incomplete oxidation of protein, and metabolizable energy was determined as the gross energy of the feed minus the energy of the feces, of the methane, and of the urine, corrected as above stated.

The data entering into the determination of the balances of matter and energy are regarded as satisfactory, except as unfavorable conditions vitiated the significance of the heat production and related data in period 13, and except for the data representing the feces in period 6. Conditions in the latter period were unsatisfactory in that it was impossible, because of refusal of feed by the subject, to have as long an experimental period as usual. The basis of separation of the intervals of preliminary feeding from the period of excreta collection was uncertain, since the quantity of feces eliminated varied greatly from day to day. In order, therefore, to arrive at the most plausible value for metabolizable energy for use in computing the net energy the preliminary feeding interval was made eight days, and the period of excreta collection six days, this separation being determined by the fact that it yielded a metabolizable energy value of corn meal in agreement with the values that were satisfactorily determined in the other experimental periods.

Seven values for heat production are given in Table 3. The heat by radiation and conduction is the heat as measured in the stream of water which carries the heat from the calorimeter chamber, diminished by the quantity of heat liberated on condensation of water on the heat absorbers.

The latent heat of water vapor was computed from the average of the daily measurements of water vapor in the air current, plus the average daily quantity liberated by condensation of water on the absorbers.

The total heat directly observed is the sum of these values for heat of radiation and conduction and latent heat of water vapor. This value for total heat directly observed was then subjected to a minor correction for heat stored in or lost from the body in the gain or loss of body substance.

The total heat indirectly computed was derived from the balances of carbon, nitrogen, and energy and represents the algebraic difference between the metabolizable energy of the feed and the energy of body increase.

A crucial test of the validity of the heat measurements is afforded by a comparison of the directly measured and the indirectly computed data, since these values are derived by entirely different methods and technic. In these experiments this agreement is remarkably good, which shows that the heat measurements must have been essentially correct, and this fact should be borne in mind in the interpretation of the final results. The agreement of the directly and the indirectly determined heat production, expressed in percentage of the direct heat, (including the values for period 13, which were finally discarded), was on an average 98.7 per cent, the extremes being 97.3 and 100.9 per cent. Since there was no significant difference between these values they were averaged, and subsequent computations are based on this average figure.

The last column of Table 3 gives this average heat measurement corrected, for comparative purposes, in accordance with the established procedure of this institute, to a standard day of 12 hours standing and 12 hours lying, in order to eliminate the effect on heat production of differences of time spent by the animals in these positions.

This correction for standing and lying was based on factors experimentally determined during the fasting periods (Nos. 1 and 2),

representing the relative rates of CO_2 production during standing and lying, in accordance with a procedure described in a previous paper (*17*). For steer A the difference in heat produced per hour of standing, as compared with that produced while lying, was 13.1 Calories, while for steer B this difference was 19.8 Calories per hour per 100 kg body weight.

The figures for heat production in periods 1 and 2 represent, respectively, the third day of fast for steer A, and the fourth day of fast for steer B. These days were selected because they were the first days during which the nonprotein respiratory quotients represented the katabolism of fat alone, thus indicating a true fasting status.

A final correction of the heat production, for differences in live weight, is indicated in the fifth column of figures in Table 5, this correction representing the differences in fasting katabolism (maintenance requirement) related to the differences in the two-thirds power of the live weights in the several periods. The effect of these corrections, therefore, is to reduce the maintenance requirements of the animals, in the various periods, to the basis of a uniform live weight.

In consideration of the contents of the alimentary tract, the values actually used in this computation to represent the body weight of the fasting animals were not those observed during fast but were the average weights obtained during the periods of maintenance immediately following fast (period 3, for steer A, and period 4, for steer B), diminished by the amount of the loss in body tissue, which was computed to be 2 kg per day (*10*). It is believed that this procedure renders the live weights more nearly comparable in regard to the "fill" than would the use of the actual weight of the fasted animals.

In applying the corrections for standing, and for differences in live weight, the average of the observed and of the computed heat production was used in all feeding periods, except period 6. In this period the observed heat production was considered more reliable—for reasons already given.

METABOLIZABLE ENERGY, HEAT INCREMENT, AND NET ENERGY VALUES OF CORN MEAL FOR BODY INCREASE

In Table 5 are given the metabolizable energy, heat-increment, and net energy values of corn meal for body increase, as influenced by the other components of the rations in which the corn meal was fed.

These values were derived by a comparison of data obtained from the basal maintenance rations with parallel observations from the production rations, which were the same as the former plus corn meal.

Three such comparisons were possible for each animal—periods 3 and 5, 7 and 9, 11 and 13, for steer A, and periods 4 and 6, 8 and 10, 12 and 14, for steer B. In each case the differences in the metabolizable energy, in the heat production, and in the gains of energy have been ascribed to the corn meal in the production rations and have been expressed in relation to kilograms of dry matter. Actually there were slight differences in the dry matter of the basal components of the rations compared, on account of slight differences in moisture content, but these differences were considered negligible in relation to this computation.

TABLE 5.—*Metabolizable energy, heat increment, and net energy values of corn meal*

Period No.	Steer	Plane of nutrition, and kind of roughage in ration	Dry matter of daily ration	Dry matter of added corn [a]	Metabolizable energy		Total heat computed to standard day and live weight	Heat increase per kilogram of dry matter of added corn	Energy balance	Net energy per kilogram of dry matter of corn added to basal ration
					Total	Per kilogram of dry matter of corn added to basal ration				
3	A	Maintenance, oat straw	*Grams* 3,954	*Grams*	*Calories* 8,767	*Calories*	*Calories* 9,233	*Calories*	*Calories* −466	*Calories*
5	A	1.5 maintenance, oat straw	5,103	1,149	12,659	3,387	10,305	933	2,354	2,454
4	B	Maintenance, oat straw	3,610		7,646		8,530		−884	
6	B	1.5 maintenance, oat straw	4,661	1,051	11,155	3,339	9,393	821	1,762	2,518
7	A	Maintenance, timothy hay	3,970		8,980		8,869		111	
9	A	1.5 maintenance, timothy hay	5,206	1,236	12,891	3,164	10,483	1,306	2,408	1,858
8	B	Maintenance, timothy hay	3,428		7,410		7,854		−444	
10	B	1.5 maintenance, timothy hay	4,530	1,102	10,656	2,946	9,288	1,301	1,368	1,645
11	A	Maintenance, alfalfa hay	4,801		10,978		10,122		856	
13	A	1.5 maintenance, alfalfa hay	6,116	1,315	14,850	2,944				
12	B	Maintenance, alfalfa hay	4,148		9,025		9,056		−31	
14	B	1.5 maintenance, alfalfa hay	5,315	1,167	12,558	3,027	10,233	1,009	2,325	2,018

[a] This value, determined as the difference between 2 rations, is very slightly in error as representing the corn meal added to the basal ration owing to the fact that while the basal ration and the basal components of the supplemented ration were identical, on the fresh basis, they differed slightly in moisture content in the 2 rations (periods) compared.

The net energy value of the corn meal is the difference between the metabolizable energy value and the heat increment value; or, it may be said to represent the difference in the gain of energy between the two periods compared, related to the difference in feed (corn meal).

This method of computation of the net energy value of a single feeding stuff, for body increase, was proposed by Armsby and was employed by him in many of his experiments. It is the simplest and most direct of the methods available for this purpose, and in this series of experiments is the only method usable. It has, however, some undesirable features which must be recognized. These have been discussed at some length by one of the writers elsewhere (*19*). The important fact to bear in mind in this connection is that in case the difference in feed consumed in the periods compared is small, this method of computation may cause extensive exaggeration of experimental errors.

The metabolizable energy values of the corn meal exhibited appreciable variation, the highest value being obtained when the corn meal was fed in combination with the basal ration containing oat straw.

The most striking results of these experiments are the very different heat increment values obtained for corn meal in the different comparisons made. Thus, for steer B the heat increment values

for corn meal in the three rations containing oat straw, timothy hay, and alfalfa hay were 821, 1,301, and 1,009 Calories., respectively, while for steer A the corresponding values for the rations containing oat straw and timothy hay were 933 and 1,306 Calories, respectively.

As a result of the variations observed in the metabolizable energy values, and in the heat increment values, the net energy values of the corn meal for body increase also vary greatly. When the corn meal was combined with the basal ration of oat straw and corn meal the net energy value per kilogram of dry matter of the added meal was found to be 2,454 Calories for steer A, and 2,518 Calories for steer B. In the timothy-hay corn-meal combinations the net energy value of the meal was found to be 1,858 Calories for steer A, and 1,645 Calories for steer B. When the corn meal was added to the high-protein basal ration containing alfalfa hay the net energy value of the meal was 2,018 Calories for steer B, and for reasons already explained there is no value given for steer A. These results, therefore, show maximum variation in the apparent net energy value of corn meal for body increase, at comparable planes of nutrition, with a single experimental subject, between 1,645 and 2,518 Calories.

These differences in metabolizable energy, heat increment, and net energy values of corn meal for body increase may be regarded mainly as results of mutual nutritive supplementation of the ration components, but also in part as results of the individuality of the experimental subjects and of arithmetical exaggeration of experimental error, the principles of which have been discussed at length (*11*).

The plan of experimentation was not such as to reveal the degrees of nutritive supplementation among ration components which were effective in these relations. Under the circumstances, it would be idle to attempt an analytical accounting for the differences in results obtained.

The foregoing observations having to do with nutrition on a production level, similar comparisons will be made of the values of the basal rations, fed on planes of approximate energy equilibrium. (Table 6.)

TABLE 6.—*Metabolizable energy, heat increment, and net energy values of the basal rations, for maintenance*

Period No.	Steer	Components of rations	Dry matter of daily ration	Metabolizable energy		Heat production, corrected	Fasting katabolism	Heat increment		Net energy per kilogram of dry matter	Utilization of metabolizable energy
				Total	Per kilogram of dry matter			Total	Per kilogram of dry matter		
			Grams	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
3	A	Oat straw, corn meal,	3.954	8,767	2,217	9,233	6,309	2,924	740	1,477	66.6
4	B	molasses	3.610	7,646	2,118	8,530	6,054	2,476	686	1,432	67.6
7	A	Timothy hay, corn	3.970	8,980	2,262	8,869	6,309	2,560	645	1,617	71.5
8	B	meal	3.428	7,410	2,162	7,854	6,054	1,800	525	1,637	75.7
11	A	Alfalfa hay, wheat	4.801	10,978	2,287	10,122	6,309	3,813	794	1,493	65.3
12	B	bran, linseed meal	4.148	9,025	2,176	9,056	6,054	3,002	724	1,452	66.7

In spite of the obviously marked differences between the nutritive values of the three basal maintenance rations, their metabolizable

energy per kilogram of dry substance was approximately the same for the three; thus the metabolizable energy values for the ration of oat straw, corn meal, and molasses for steers A and B were 2,217 and 2,118 Calories, respectively; for the timothy-hay and corn-meal ration the corresponding values were 2,262 and 2,162 Calories; and for the ration of alfalfa hay, wheat bran, and linseed meal the values were 2,287 and 2,176 Calories.

The heat increments per kilogram of dry matter of ration also agreed closely. These values for steers A and B, respectively, were, for the oat-straw and corn-meal ration, 740 and 686 Calories; for the timothy-hay and corn-meal ration, 645 and 525 Calories; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 794 and 724 Calories.

The net energy values per kilogram of dry matter of these rations for maintenance were, for steers A and B, respectively, for the oat-straw and corn-meal ration, 1,477 and 1,432 Calories; for the timothy-hay and corn-meal ration, 1,617 and 1,637 Calories; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 1,493 and 1,452 Calories.

Further, the utilization of the metabolizable energy of these three basal maintenance rations by steers A and B, respectively, were, for the oat-straw and corn-meal ration, 66.6 and 67.6 per cent; for the timothy-hay and corn-meal ration, 71.5 and 75.7 per cent; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 65.3 and 66.7 per cent.

These data reveal the fact that the two steers were in remarkably close agreement in capacity to utilize the three rations, but that each of the rations exhibited a higher metabolizable energy, a higher heat increment, and a lower percentage utilization of the metabolizable energy for steer A than for steer B.

In the light of these observations it is highly probable that the net energy value of the corn meal, for body increase, for steer A in the unsuccessful period 13 would have been essentially the same as was determined for steer B in period 14, if the conditions governing the heat production in the former case had not got out of control.

It is regrettable that the diversity which characterizes net energy values of individual feeding stuffs can not be factored in such manner as exactly to separate the complex combinations of contributing influences, and to assign each to its cause or source; but the situation is so exceedingly complicated that the best that can be done at this time is to study these influences, one at a time, in specially devised and elaborately controlled investigations, and thus demonstrate their existence, and the ways in which and the prominence with which they affect results.

The general significance of the results of this study, therefore, is to tend to support the conclusion of Forbes (7) that foodstuffs express their characteristic net energy values only as they are components of nutritively complete rations; which is equivalent to the position that net energy values of individual feeding stuffs are not constants; also, in this light, such values seem not to be characterized by accuracy commensurate with the particularity, the laboriousness, and the expense of directly determining them.

Intensive research on the energy metabolism of farm animals may be fully justified, however, by the determination of principles of

general applicability; and such principles may be of immense service to economic interests, even though compromises are made in utilizing them which are limited, in degree, only by the desirability of the immediate economic results.

The practical consequences of this conclusion are discussed in a paper by Forbes (*8*).

SUMMARY

The associative or supplementing effects of feeding stuffs in combination were studied by respiration calorimetry in a duplicate series of observations with two 2-year-old steers as experimental subjects, the specific object of the investigation being to learn whether the net energy values of feeding stuffs are affected by the nutritive balance of the combinations in which they are fed during 28-day periods of experimentation—in other words, to learn whether net energy values of individual feeding stuffs are constants.

Energy-balance determinations were made with each of the steers during fast, at the energy-maintenance level with three rations of different character, and at a production level, the rations being the same as the three fed at the maintenance level, plus corn (maize) meal equivalent to half of the estimated energy-maintenance requirement.

The three basal rations differed widely as to protein, mineral, and presumably as to vitamin contents and therefore differed in their capacities to supplement corn meal.

Heat production was measured by direct calorimetry, checked by indirect measurement.

The apparent digestibility of the corn meal differed decidedly as affected by the other constituent feeding stuffs of the rations in which it was fed and therefore affected the net energy values determined.

The apparent metabolizable energy values of corn meal, fed on a production level, differed between 2,946 and 3,339 Calories per kilogram of dry matter in the case of one steer, and between 2,944 and 3,387 Calories in the case of the other, as affected by the rations in which the corn meal was fed.

The apparent net energy value of corn meal, fed on a production level, differed between 1,645 and 2,518 Calories per kilogram of dry matter, in the case of one steer, as affected by the rations in which the corn was fed.

The average of the three lowest determinations of the digestible crude protein maintenance requirement of these steers was 21.7 g per 100 kg of live weight per day.

The ratios of carbon to nitrogen in the urine were 1.2, 1.2, 1.3, and 1.3 to 1 on high-protein rations, and 3.6, 3.2, 3.2, 3.7, 3.3, 3.8, 3.9, and 4.0 to 1 on low-protein rations.

The results tend to support the conclusion of Forbes that feeding stuffs express their characteristic net energy values only as they are components of nutritively complete rations.

Individual foodstuffs, it seems, are susceptible of only superficial evaluation apart from the rations in which they are used.

It is necessary, therefore, to redirect the interest of the student and of the practical animal husbandman to energy values of rations as wholes, especially of nutritively balanced rations, rather than to such values of their constituent feeding stuffs.

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